

132

Acc. No. 167
GXI, 87 NE

Page No.	Date of Issue
257	26-12-54
298	12/1/55
342	12/8/55
161	3-1-55
149.	8.4.60
94	7/11/60 7/11/60.
—	29-7-65 15-7-65
—	13/11/51 5/11
—	1/6/69 18/5
—	4.6.70 22/5

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Monographs of the Pickett-Thomson Research Laboratory
Volume I

THE VITAMINS

By

ETHEL BROWNING

M.D. (LIVERPOOL)

ASSISTANT PATHOLOGIST TO THE PICKETT-THOMSON RESEARCH LABORATORY
ST. PAUL'S HOSPITAL, LONDON



PUBLISHED FOR

THE PICKETT-THOMSON RESEARCH LABORATORY
ST. PAUL'S HOSPITAL, 24 ENDELL STREET, LONDON, W.C. 2

BY

BAILLIÈRE, TINDALL & COX

7 AND 8 HENRIETTA STREET, COVENT GARDEN, LONDON, W.C. 2

IN AMERICA

THE WILLIAMS AND WILKINS COMPANY
BALTIMORE, U.S.A.

1931

PRINTED IN GREAT BRITAIN

167 ✓

GXI,87

N31

PREFACE

THE *Annals* of the Pickett-Thomson Research Laboratory have so far dealt only with Bacteriology, and it is our ambition that they should build up into an ever-increasing encyclopædia and atlas on this science.

This work on *The Vitamins* by Dr. Ethel Browning will form the beginning of a new series of volumes, which will be entitled the "Monographs of the Pickett-Thomson Research Laboratory." It is hardly necessary to say that these volumes will also give a very complete review of the subjects dealt with up to the time of their publication.

Even a cursory examination of Dr. Browning's work is sufficient to reveal the extraordinary thoroughness with which she has handled this difficult subject. It is a splendid edifice of mental toil, entailing years of continuously sustained and tireless labour.

No one who has not attempted to produce a volume of this kind can possibly conceive the arduous nature of such a task. It can be achieved only by a combination of superb human qualities: unusual intelligence, indomitable perseverance, and a capacity for long sustained concentration, combined with great patience and endurance. To these must be added a great longing after the truth, and a heroic desire to help mankind, before magnificent work of this kind can be accomplished without hope of adequate reward.

One would expect that a robust constitution was a necessary adjunct to these qualities in order to support the mind in the years of incessant labour required in great compilations of this nature. Dr. Ethel Browning has proved, however, that even in a delicate constitution mental powers can surmount all difficulties.

With these few inadequate remarks I have tried to convey to the reader the impression of the years which she has devoted to this volume. I have watched these years of relentless toil with anxiety, feeling responsible for encouraging her in a task which might perhaps have proved too great a strain upon her health.

I hope, therefore, that those who study this volume will not only admire, but appreciate, the importance of her contribution to science, since she has successfully organised the almost inextricable tangle of information which has accumulated during the last twenty to thirty years.

Hundreds of workers are producing papers which record the results of numerous experiments, but very few are able to face the task of the endless study and sifting necessary to organise the heterogeneous mass of some three thousand papers into a well-ordered volume of information. If we assume that each paper represents on the average one year of investigation, then this volume gives the epitomised knowledge from three thousand years of research.

The world is crying out to-day for great compilations of this kind, because with the ever-increasing flow of research papers turned out annually by thousands of workers, the mind is overwhelmed in the great tidal wave of knowledge, until some person with infinite patience and skill is able to compile a complete and thoroughly indexed work on a definite subject such as Dr. Browning has done.

D. THOMSON.

August 1931.

INTRODUCTION

THE original purpose of this monograph was to collect into one volume as much as possible of the existing literature on the subject of Vitamins, and to present to the scientific reader a comprehensive account of the researches which have established the vitamin theory on its present basis. That purpose has been followed as strictly as limitations of time and space would allow, but the literature on the subject has assumed such colossal proportions, while opinions, even amongst those who have devoted years of study to only one aspect of the vitamin problem have proved so divergent, that the author has had perforce to remain content with the endeavour to weave into a complete pattern those threads which have seemed most important to the continuity of the design.

During the last few years, great attention has been focused on the attempt to isolate vitamins as chemical entities. The biochemistry of the vitamins has therefore become a subject of world-wide interest, and it might be expected that any new volume dealing with the vitamins would tend to develop into a biochemical treatise. There has been, in the present monograph, an attempt to neutralise this tendency to some extent, by devoting a good deal of attention to the clinical and experimental aspect, which has, in the author's opinion, become somewhat overshadowed during the recent period of chemical activity. For it is, after all, in the preventive and therapeutic possibilities of vitamin administration that their ultimate importance to humanity in general will consist, and though it is desirable and necessary that the biochemist should know the exact chemical constitution of the substances with which he is dealing, it is equally desirable and necessary that the physiologist and clinician should know the exact effect of these substances on living tissue in health and disease. There are indeed some students of the vitamin problem who believe that the conception of vitamins as chemical entities is not established. The views of Baly, that vitamins can be expressed in terms of high energy, and of von Hahn, that the water-soluble factors, at any rate, can be expressed in terms of surface tension, are illustrative of this point of view.

The classification of the vitamins, according to the symptoms and structural changes produced in the animal organism by their complete absence, has led to a closer examination of the less defined effects of the nutritive failure due to their insufficiency. That such nutritive failure has been dependent until very recently, not so much upon economic inability to procure vitamin-containing foods as upon lack of knowledge on the part of the public as to where the respective vitamins are to be found, and how preserved, during the modern handling of food-stuffs, is shown by the recent impetus given to the advertising of vitamin-rich products. As an American writer has truly observed (*Journ. Amer. Med. Assoc.*, 1931), "modern advertising has all too often presented a dire picture of vitamin starvation among the population, and has ventured to arouse the fear of dire consequences unless immediate steps are taken to supply the lacking essential." Nor have the commercial exponents of vitamins, at least until the very definite discovery of the toxic effects of overdosage of vitamin D made it impossible to ignore such pathological consequences, taken into sufficient consideration the question of hypervitaminosis. It is to be hoped that this question, of which several aspects remain under discussion, will soon be elucidated, for not until the possibilities of hypervitaminosis and the

means of its avoidance rest on a scientific basis can the field of vitamin therapy be successfully explored.

The first part of the monograph has been devoted to a consideration of the vitamins from a more or less non-specific point of view. There is at present a certain section of workers who emphasise this aspect of vitamin action, especially when considering their therapeutic possibilities. These workers believe that more benefit will eventually be derived from the use of "vitamin-rich" diets, that is, of diets consisting of a variety of fresh foods, than from the separate administration of foodstuffs, or extracts of foodstuffs, in which one or other vitamin predominates.

In dealing with each vitamin under its separate heading, however, the widely accepted classification of each according to its connection with specific disorders of health has been followed. Opinions are not entirely unanimous as to the absolute specificity of the "anti-infective" action of vitamin A, or the exact degree of connection between vitamin B₁ and beriberi, or between vitamin B₂ and pellagra, and such a vast amount of information is now available on these subjects that a very comprehensive bibliography is a necessary adjunct to a résumé, however extensive, of the chief papers. It is for this reason that many papers not mentioned in the text are referred to in the bibliography. By this means it is possible to apply a high-power magnification to any detail of the particular subject under consideration.

In the table of the Vitamin Values of Foodstuffs it will be seen that investigations made in the light of recent extensions of classification and improved methods of biological estimation have considerably modified some of the values given by early workers. So far as the author knows, no other existing table includes the vitamin B₂ and E content of foods. The values for vitamin E, here given, are based chiefly on information derived from the comprehensive monograph of Evans and Burr (*California Univ. Mem.*, 1928, vol. 8).

The values of canned foodstuffs so far estimated throw an interesting light on the development of the canning industry. According to these estimates, modern methods would appear to have removed the danger of devitaminising foods during the process of preservation. The estimates are, of course, based on animal experiments, and in some quarters it is held that these experiments cannot strictly be applied to human beings. In view of the widespread interest in the response of human disorders of health to "vitamin-rich" diets and of the almost unavoidable necessity for the inhabitants of large towns to depend to some degree on preserved foodstuffs, a comparison of the effects of human dietaries composed largely of fresh and home-cooked foods and of those containing a large proportion of canned foods would be of incalculable value to the community.

The science of nutrition has made enormous strides since the conception of food in terms of protein, fat, carbohydrate, and calories has embraced also its vitamin value. It can only make further advances when a great effort is made to correlate the scattered knowledge into a harmonious whole. It is hoped that this volume will play its part in such an epitome of knowledge, so far as the vitamins are concerned.

ETHEL BROWNING.

August 1931.

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THE VITAMINS

PART I

THE VITAMINS IN GENERAL

I. HISTORICAL SKETCH OF THE DISCOVERY OF VITAMINS.

For the following details of the discovery of vitamins the author is largely indebted to the account given in the Medical Research Council Report (Special Report Series, No. 38, reprinted 1928).

(a) **DISCOVERY OF "ACCESSORY FOOD SUBSTANCES."**—The first suggestion that other substances in addition to the recognised dietary units of proteins, carbohydrates, and salts, which were equally indispensable for life, came from Lunin, as far back as 1881. The anti-scurvy value of lemon juice was none the less well known to the British Navy in 1800, and Chartier, as early as 1535, had found a fresh extract of pine needles efficacious against outbreaks of scurvy amongst his crew.

In 1881 Lunin was investigating the significance of inorganic salts in the nutrition of animals. He found that his experimental animals, while capable of living and maintaining health on a diet of milk, were unable to live on the separate ingredients of milk—caseinogen milk fat, milk sugar, and the ash of milk.

Commenting upon his experiments, he remarked : " Mice can live quite well under these conditions when receiving suitable foods (*e.g.* milk), but as the above experiments demonstrate that they are unable to live on proteins, fats, carbohydrates, salts, and water, it follows that other substances indispensable for nutrition must be present in milk besides caseinogen, fat, lactose, and salts."

This supposition of Lunin was taken up by Bunge, in 1892, who, in his *Cours de Chimie biologique et pathologique*, emphasised the question : " Are there other substances in milk besides fats, albuminoid matter, and carbohydrates necessary to the vital processes ? " " Il serait utile," he remarks, " de continuer ces recherches."

Other early attempts were made by Socin (1891), Pasqualis (1895), Jacob (1906), Salta and Noegerrath (1906), McCollum (1909), and others to nourish animals on diets composed of purified proteins, fats, carbohydrates, and salts, and many theories were advanced to explain the repeated failures. Monotony of diet, with consequent failure of appetite, was suggested : a theory negatived by the fact that animals were found to maintain normal nutrition upon diets consisting of single foodstuffs, such as milk or egg yolk. Nor could unpalatability be regarded as the cause of nutritive failure, for the addition of flavouring substances to the synthetic ration made no difference to the ultimate decline of the experimental animals.

One research worker, Rohman (1903, 1908), claimed to have been able not only to maintain mice in health on diets consisting of a mixture of isolated and " purified " components, but to ensure increase of weight and even reproduction. In the light of subsequent researches it would seem probable that the purification of his food substances had not been carried out with sufficient thoroughness to destroy the constituents now known to be necessary for satisfactory maintenance.

As early as 1906 Hopkins wrote : " But, further, no animal can live upon a mixture of pure proteins, fats, and carbohydrates, and even when the necessary inorganic material is carefully supplied, the animal still cannot flourish. The animal body is adjusted to live either upon plant or other animals, and these contain countless substances other than the proteins, carbohydrates, and fats. Physiological evolution, I believe, has made some of these well-nigh as essential as are the basal constituents of diet ; lecithin, for instance, has been

repeatedly shown to have a marked influence upon nutrition, and this just happens to be something familiar, and a substance that happens to have been tried. The field is almost unexplored, only it is certain that there are many factors in all diets, of which the body takes account.

“ In diseases, such as rickets, and particularly scurvy, we have had for long years knowledge of a dietetic factor, but though we know how to benefit these conditions empirically, the real errors in the diet are to this day obscure. They are, however, certainly of the kind which comprises these minimal qualitative factors that I am considering. Scurvy and rickets are conditions so severe that they force themselves upon our attention, but many other nutritive errors affect the health of individuals to a degree most important to themselves, and some of them depend upon unsuspected dietetic factors.”

In the meantime, work had been proceeding in another field, which eventually led to the description of a chemical entity of a basic nature by the word “ Vitamine.”

Eijkman, in 1897, had shown that polyneuritis could be induced in fowls by restricting them to a diet of polished rice, and that a diet of undecorticated rice would cure fowls in this condition. His statistics dealt with no fewer than 279,621 individuals, all of them at different times prisoners in the gaols of the Dutch East Indies. The different prisons, owing to their different situations, with different and settled local customs, were supplied with different kinds of rice, so that the conditions for controlled observations were ready to hand.

In thirty-seven prisons unpolished rice was employed ; only one of these prisons developed cases of beri-beri. In thirteen prisons the rice used was polished rice mixed with unpolished ; in six of them beri-beri developed. Out of fifty-one prisons, where the rice eaten was entirely of the polished variety, as many as 36, or over 70 per cent., developed cases of the disease. From these data it is apparent that for each 10,000 of the prison population there was only 1 case among those eating unpolished rice, 416 on the mixed rice dietary, and no less than 3900 on polished rice.

Eijkman's conclusions were further confirmed by Braddon (1907, 1909) as the result of epidemiological investigations extending over 10 years. Braddon's work brought out the further important point that natives consuming rice which had been parboiled before the removal of the pericarp did not contract the disease. As the result of the substitution of parboiled for polished rice based on his recommendations, the case mortality of beri-beri in the hospitals of the Malay States was greatly diminished.

Grijns (1901, 1909) showed that foodstuffs other than unpolished rice could prevent polyneuritis, but he found that their protective properties were destroyed by heating to 120° C. He put forward the view that beri-beri results from a deficiency in polished rice of a substance necessary for the metabolism of the nervous system.

In the Philippines the subject had been under extended investigation by a number of workers.

In 1909 Fraser and Stanton showed that the alcohol extracts of rice polishings would relieve experimental polyneuritis, and in 1911 they ascertained that polished rice contained less phosphorus than unpolished rice, and that the content of phosphorus was a useful indication as to whether the rice was an adequate diet or not. These results supported Schaumann's theory (1908) that beri-beri was caused through a deficiency in the dietaries of organic phosphorus compounds. He found (1910), however, that many phosphorus-containing substances were ineffective in preventing and curing polyneuritis ; and Chamberlain and Vedder (1911) subsequently showed that a curative extract of rice polishings contained only a minute proportion of the total content of phosphorus in the original polishings. Schaumann (1911) therefore modified his theory and suggested that the curative substance was an activator in metabolism, favouring the assimilation of certain nutrients, probably phosphorus compounds.

In 1911 Funk took up the study of the problem. Working with Cooper he found that exclusive diets of various pure carbohydrates induced polyneuritis in birds, thus disproving any intoxication hypothesis concerning the causation of beri-beri, and showed that pressed yeast, hydrolysed with 20 per cent. sulphuric acid for 24 hours, retained its property of curing polyneuritis when administered to birds.

Cooper and Funk also showed that the curative substance was entirely precipitated by phosphotungstic acid from an aqueous solution of an alcoholic extract of rice.

The earlier methods of preparation aimed at establishing the properties and chemical identity of the vitamin. The results of Funk's experiments in this direction were summarised in 1915 and 1916. He stated his original method to be as follows :

Rice polishings were extracted with cold absolute alcohol, which was partially saturated with gaseous HCl. The extracts were evaporated *in vacuo* at a low temperature, and the fatty residue melted and extracted with water. These aqueous extracts were precipitated with 50 per cent. phosphotungstic acid solution after addition of sulphuric acid to the extent of 5 per cent., and the precipitates decomposed in the usual way with baryta. The solution, entirely freed from baryta and sulphuric acid, was filtered and the filtrate neutralised with HCl and evaporated *in vacuo*. The residue was extracted with alcohol and the solution freed by filtration from the inorganic chlorides. The alcohol solution was then precipitated with alcoholic mercuric chloride solution. The active substance was found to a small extent in this precipitate, but the bulk was in the filtrate. From each of these fractions vitamin could be completely thrown down by use of silver nitrate and baryta. From this fraction after decomposition with hydrogen sulphide, there was isolated a very small quantity of a crystalline substance with a melting-point of 233°C . This was not recrystallised, and possessed very marked curative power, 0.02 to 0.04 gram curing neuritic pigeons. He ascribed to it the provisional formula $\text{CH}_7\text{H}_{20}\text{N}_2\text{O}_7$. He had already isolated curative substances melting at a similar temperature from ox brain, yeast, and milk. The active substance was precipitated by silver nitrate and mercuric acetate, but not by mercuric sulphate or nitrate; its aqueous solution was of neutral reaction, and did not react with acids. These properties suggested to Funk that the substance was probably a pyrimidine base, analogous to thymine and uracyl and a constituent of nucleic acid. In a paper (1911) he showed furthermore that certain purin and pyrimidine substances exert a marked action on pigeons affected with polyneuritis, in some cases prolonging the lives of the birds, but in others actually ameliorating the symptoms. Cooper (1913) also found that strychnine prolonged the lives of neuritic pigeons.

Edie, Moore, and collaborators (1912) had also obtained from yeast a curative substance to which they ascribed the formula $\text{C}_7\text{H}_{17}\text{N}_{21}\text{O}_5$, and Suzuki and co-workers (1912), working independently of Funk, announced simultaneously the preparation of an active substance, precipitable by phosphotungstic acid, and therefore an organic base, which Funk named "vitamine," with the idea that it was a pyrimidine base of vital importance.

That his view was not universally accepted is shown by the following statement made by W. O. Halliburton (1914) :

"Funk's view that these are pyridine derivatives and are the long-sought vitamins must be accepted with caution; the more these materials are purified the less appears to be their physiological activity in combating neuritic conditions; it is therefore exceedingly doubtful whether workers have as yet been successful in isolating the really active substance or substances.

"The very term vitamine is itself a source of fallacy, especially if the word vital is used as its adjectival form. Vital may mean one of two things: it may be used in its literal sense as equivalent to living, or it may be used in a semi-figurative sense and mean necessary or essential. When we say that vitamins are vital constituents of diet we ought to mean that

they are indispensable, and not imply that the vitamins possess the characters connoted by the word 'life.'"

In the year 1911 Osborne and Mendel published in America a monograph in which they described experiments on the nutrition of rats over considerable periods upon diets composed of isolated food substances. They used dietaries containing highly purified proteins, together with starch, sugar, lard, agar, and inorganic salts. They administered the inorganic salts either in the pure crystalline form or as a product which they called "protein free milk." This product was prepared by evaporating down milk after removal of the fat and proteins. It therefore contained the salts, lactose, and extractives of milk. With regard to these experiments, they remarked: "Although these apparently successful experiments indicated that the combinations of isolated foodstuffs employed satisfied the nutritive requirements of the rats, and consequently constituted a complete food for the maintenance of mature animals, a prolongation of the observations has led to a less favourable outcome. A continuation of the experiments over longer periods has shown that in every case, sooner or later, the animal declined; and unless a change in the diet was now instituted within a comparatively short time the animals died."

Some of the first experimental work on the importance of lipoidal substances with diet was done by Stepp (1909, 1912). He showed that certain foods, such as bread and milk, while forming ordinarily a satisfactory diet for mice, were no longer adequate to support life after extraction with alcohol and ether. He made a number of experiments in order to ascertain the nature of the essential factor removed by the extraction process, believing it to be of the nature of a lipid. The addition of the ash to the extract, or of certain neutral fats in a pure condition, did not remedy the deficiency, but he found that milk and egg yolk did. This led him to suggest the existence of an unidentified indispensable dietary unit, which he appears to have regarded as a member of the lipid class. He was unable to identify this substance with cholesterol, lecithin, kephalin, and cerebrone, since all members of that class of substance failed to restore the nutritive value of the extracted foodstuffs. He remarks in one of his papers: "It is not impossible that unknown substances indispensable for life go into solution with the lipoids, and that the latter thereby become what may be termed carriers for these substances."

In 1912 Hopkins, in England, undertook a series of experiments as a result of which he suggested the term "accessory food substances." He fed young rats upon an artificial food mixture containing caseinogen, starch, cane sugar, lard, and inorganic salts. When these constituents were given in their crude condition, they were apparently adequate for life and a certain amount of growth. When, however, they were subjected to careful purification, growth invariably ceased within a comparatively short time, and the experimental animals died. By estimating the consumption of energy of the animals, Hopkins was able to show that this failure was not due to an insufficient food intake. They ceased, in fact, to grow at a time when they were consuming food in more than sufficient quantity to maintain normal growth. In another series of experiments a very small daily allowance of milk was added to the basal ration of purified foodstuffs. In all cases this addition of milk, although the total quantity of its solids amounted to only 4 per cent., or less of the whole ration consumed, ensured normal and continual growth.

The accompanying figures (Figs. 1 and 2), reproduced from the Medical Research Council Report (Special Report Series, No. 38), show the effects of the addition of small quantities of milk (2 to 3 c.c. per day) to the synthetic diets. Similar effects were observed from the addition of protein-free and salt-free extracts of milk solids or of yeast. Hopkins' own conclusions from the results of these experiments were as follows:

"It is possible that what is absent from artificial diets and supplied by such addenda

as milk and tissue extracts is of the nature of an organic complex (or of complexes) which the animal body cannot synthesise. But the amount which seems sufficient to secure growth is so small that a catalytic or stimulative function seems more likely.

"It is probable that our conception of stimulating substances may have to be extended. The original vague conception of such subjects being condiments, chiefly affecting taste, gained in definiteness by the work of the Pavlov School. But the place of specific diet constituents which stimulate the gastric secretory mechanism can be taken by the products of digestion itself, and in this connection the stimulant in the diet is by no means indispensable. Most observers agree that the addition to normal dietaries of meat extracts capable of stimulating the gastric flow does not increase the actual absorption of food, though this point could be properly tested by adding them to an artificial dietary known to be free from analogous substances. As was emphasised above, the milk did not affect absorption in my experiments. But such undoubted stimulating effects due to diet constituents as those discovered by Pavlov may quite possibly be paralleled elsewhere in the body on more specific and indispensable lines. Stimulations of the internal secretions of the thyroid and pituitary glands which are believed on very suggestive evidence to play an important part in growth processes, can be legitimately thought of. On the other hand, the influence upon growing tissues may be direct. *If the attachment of such indispensable functions to specific accessory constituents of diets is foreign to current views upon nutrition, so also is the experimental fact that young animals may fail to grow when they are absorbing daily a sufficiency of formative material and energy for the purpose of growth.*"

Following up their experiments with the "protein-free milk," Osborne and Mendel now showed that the milk fat, as well as the "protein-free residue, had the same growth-promoting effect as Hopkins' accessory factors," while the purely artificial diets still failed to ensure maintenance. They were at this time unable to decide "whether the deficiency of the purely artificial diet is to be attributed to improper proportions of its constituents, to improper combinations of these constituents, or to the lack of some essential element." They stated, however (1912), that they had had a "considerable degree of success" in feeding in the absence of the "hypothetical organic hormones," and attributed this success to the fact that the salt mixtures which they used closely resembled the salts of milk. Hopkins and Neville (1913), realising that these results, if indisputable, would imply that the "accessory factors of

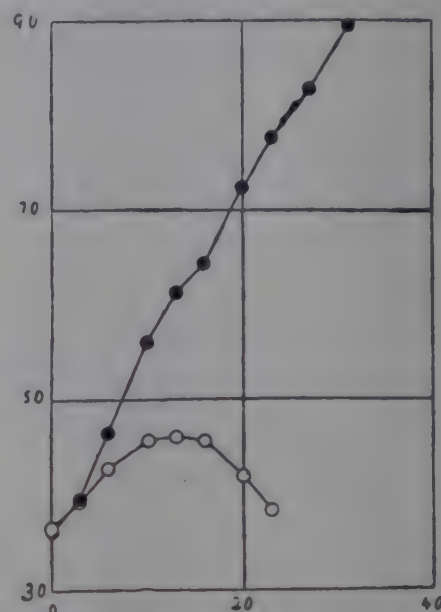


FIG. 1. Lower curve, six rats on artificial diet alone. Upper curve, six similar animals receiving in addition 2 c.c. of milk each per diem. Abscissae, time in days; ordinates, average weight in gram.

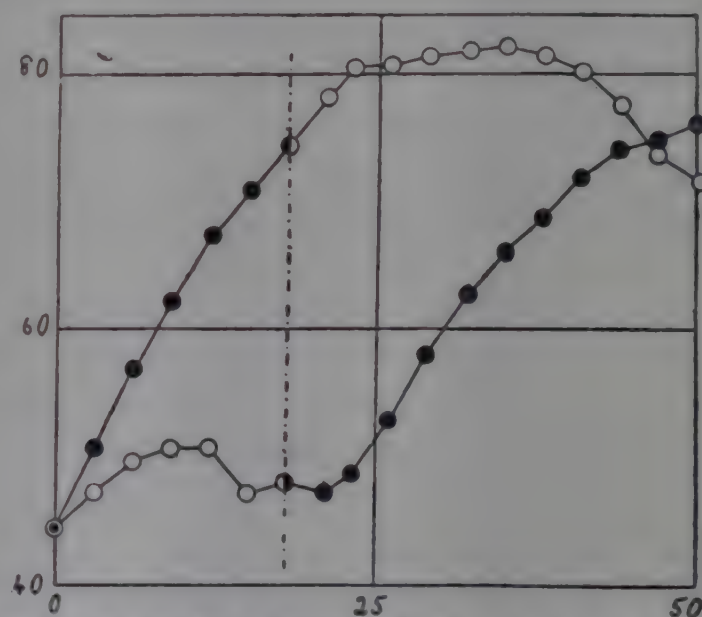


FIG. 2. Lower curve (up to eighteenth day), eight male rats upon pure dietary; upper curve, eight similar rats taking 3 c.c. of milk each a day. On the eighteenth day, marked by vertical dotted line, the milk was transferred from one set to the other. Average weight in gram, vertical: time, horizontal.

uncertain nature" were not indispensable, attempted to repeat Osborne and Mendel's experiments. They used the same salt mixtures, and the same ingredients, purified with extreme care, but were unable to obtain any appreciable growth on such diets, and death of the experimental animals invariably took place within 40 days. At the same time they were able to confirm Hopkins' earlier results in that 2 c.c. per day of milk added to the basal diet was found to bring about resumption of growth. The existence of these "complex lipoids," "vitamines," or "hormones," indispensable for growth and maintenance was therefore to be considered as an indisputable fact.

(b) **DIFFERENTIATION OF TWO ACCESSORY FACTORS.**—The fundamental facts of the necessity of certain non-artificial substances had already been confirmed also by McCollum and Davis (1913). The basal diet used by these workers, and which they found inadequate for maintenance, included purified casein, carbohydrates (lactose, starch, dextrin), lard, and agar. McCollum and Davis made many tentative attempts to render this diet satisfactory, and discovered that while the addition of an ethereal extract of eggs or butter was successful, lard and olive oil gave no result.

They concluded that the ether-soluble extracts of egg-yolk and butter contained certain organic complexes without which the animals could not grow, though they could be maintained in fairly good health for extended periods. They thought it possible that the necessary substances might be either phosphatides or foreign bodies accompanying them. Osborne and Mendel (1914) now took up a more detailed investigation of the growth-promoting properties of the different fats. They found that while a diet composed of edestine, starch, butter fat, lard, and protein-free milk would allow maintenance and reproduction to the third generation, replacement of the butter fat portion by an equal weight of lard led to the death of the animals in 90 to 120 days. Neither olive oil nor cotton-seed oil was able to cause resumption of growth, and incidentally they observed that the loss of weight was often accompanied by an "infectious eye disease." It was apparent from these experiments that all fats had not the same nutritive value, and that some contained substances, or possessed properties, of which others were devoid. Later (1916), Osborne and Mendel located the active principle in the butter fat fraction of the butter, and since this fraction was free from nitrogen and phosphorus and contained no water-soluble or ash-yielding substances they considered that the accessory factor in question could not be identical with the water-soluble factor postulated by Funk. The latter worker, however, like Hopkins and Neville, doubted the complete purification of their butter fat, and claimed to have extracted nitrogen-containing substances from their preparation.

In the meantime, other workers had been finding that even butter fat added to a ration of purified substances did not ensure completely satisfactory nutrition. Thus, Funk and Macallum (1915) found that young rats not only failed to grow and eventually died on such a diet, but also showed symptoms prior to death which resembled those of avian beri-beri. When yeast was added, however, growth was at once resumed, whereupon they stated "that the growth-promoting factor is beyond question contained in yeast."

It remained for McCollum and Davis (1915) to make the actual assumption that two distinct essential factors must exist in natural foodstuffs. In studying the dietary deficiencies of mice, they found that while the addition of purified casein, of a "growth-promoting" variety of fat, and of mineral substances did not render it a completely satisfactory food, the addition of small quantities of wheat germ or milk powder furnished a sufficient quantity of some other substance whose presence was essential for normal growth. They called this substance "water-soluble B" in contradistinction to the "fat-soluble A" present in fats. At this time their observations on lactose cleared up a discrepancy in some of the results then in evidence. McCollum and Davis (1915) showed that lactose, unless purified by repeated

crystallisation, was "contaminated" by traces of a nitrogenous substance, which, like the unknown factor present in egg-yolk and butter fat, played an essential part in growth. Many of the earlier workers had included lactose, insufficiently purified, in their synthetic rations, and had therefore provided at any rate a small quantity of the water-soluble factor.

Funk and Macallum had excluded lactose from their constituents on account of its content of traces of nitrogenous products, and were therefore unable to obtain normal growth until they supplied the water-soluble factor in the form of yeast.

These results were confirmed by Drummond (1916) who brought forward evidence of the "impurity" of commercial lactose, and also of the "protein-free" milk of Osborne and Mendel.

Most of the workers on the water-soluble vitamin had employed rats as their experimental animals, but researches on the effects of artificial diets were now extended to include pigeons. Funk and Schonborn (1914), McCollum and Kennedy (1916), and others found that lack of the constituent which had been found by McCollum and Davis in rice and wheat germ, and by Funk and Macallum in yeast, produced symptoms which are now known as those of avian polyneuritis. By 1919 the existence of the two separate factors was almost universally recognised, with the exception of a small minority who were in agreement with the views of Rohman, expressed in 1916. Rohman still claimed to be able to maintain growth on artificial diets, and refused to admit the necessity of assuming the existence of any unknown factors indispensable for growth.

Osborne and Mendel, however, on making careful examination of their own account of their experiments, were able to show that many of their "artificial diets" were so incompletely purified that they must have contained "some or all of the other constituents of milk among which are those proved to be especially efficient in promoting growth." The connection between the fat-soluble vitamin and the occurrence of the eye disease known as xerophthalmia was demonstrated by several observers between 1915 and 1920. That such a condition was associated with some defect in nutrition had been observed as early as 1857 by McKenzie, and various outbreaks of epidemics in industrial schools had been reported from 1880 onwards. Such an outbreak was that reported by McGowan and McNeil (1913). During an epidemic of what they called "distorted" pneumonia, these observers drew attention to the prevalence in this school of a chronic granular conjunctivitis, which they clearly recognised as non-contagious.

At that time the existence of the fat-soluble vitamin was not clearly proved, but when the next outbreak occurred in the same industrial school in 1919, Findlay, who investigated the matter, observed the resemblance of the eye condition to the experimental keratomalacia, now known to be produced by vitamin A deficiency.

In the meantime, Mori, as early as 1904, had described the ocular complications of the disease known in Japan as "Hikan," and had found that the administration of cod-liver oil cured them, though not the other symptoms, and that sesame oil and olive oil were inefficacious.

Cases of eye infection were also described by Bloch (1912, 1917) in Denmark. In an institution the occurrence of the disease was limited to a group of children who received no butter, cream, or eggs, and only half-skimmed milk. When whole milk and cod-liver oil were given the symptoms disappeared. Other workers, particularly Blegvad (1923, 1924), recorded numerous cases of an xerophthalmia traceable to a deficiency of the fat-soluble vitamin A, and curable by cod-liver oil.

(c) **DISCOVERY OF A SECOND WATER-SOLUBLE VITAMIN.**—The existence of another accessory factor, allied to the incidence of scurvy, as water-soluble B, was now believed to be allied to the incidence of beri-beri, and fat-soluble A to that of xerophthalmia, appeared probable from the results of many investigations into the disease of scurvy. The connection between

infantile scurvy and boiled milk had been observed in 1894 by Barlow, who stated that he believed the antiscorbutic quality of milk was lessened in some degree by boiling and prolonged sterilisation.

Meyer (1901), Neumann (1902), Heubner (1903), and others also ascribed infantile scurvy to the consumption of diets consisting chiefly of boiled milk, and concluded that the deficiency of some accessory substance in the diet was the cause of the symptoms described.

The experience of early navigators, such as Cook, Jackson, and Harley (1900), and many others, also pointed to the existence in fresh fruits and vegetables of some substance which prevented the occurrence of scurvy among their crews. It was not, however, until Holst (1907) was invited to investigate the feeding of Norwegian soldiers that the actual link between experimental and human scurvy was made.

Holst and Frölich (1907–1912) pointed out that symptoms resembling those of scurvy could be induced in guinea-pigs and rabbits by exclusive diets of barley, oats, rice, and bread, and prevented by the addition to these diets of sufficient amounts of various antiscorbutic food-stuffs such as green vegetables, fruit, etc.

Fürst (1909) also made the interesting observations that yeast, which was very effective in preventing beri-beri, possessed no protective properties against scurvy, and that whereas grain was lacking in the essential principle, it developed an antiscorbutic substance during germination. He suggested that this substance is produced in the plant by the agency of enzymes.

Frölich (1912) had also shown that pasteurised milk (that is, milk heated to 70° C.) prevented the development of scorbutic symptoms in guinea-pigs fed on oats, while milk heated at 98° C. for 10 minutes did not.

Exclusive diets of either raw or boiled milk, however, did not induce scurvy. Cooper, writing in 1913, remarked: "It would appear that the amount of antiscorbutic substance normally present in an average sample of milk is such that, although some is destroyed by heating to the above extent, there is still sufficient left to supply the needs of the infant's organism. The fact that infantile scurvy does occasionally result from diets of boiled milk may be due to the particular milk being poor in the active substance, or to the requirements of the children being greater than usual, or possibly, as suggested by Frölich's researches, to the addition of starchy foodstuffs to the diet."

This "active substance" was found by Holst and Frölich (1911, 1912) to be soluble in acid, insoluble in petrol, ether, and dialysable. In 1913 Funk found that milk retained its antiscorbutic properties after the elimination of the proteins, and that its active constituent, which he did not hesitate to classify among the "vitamins," was carried out of solution by kaolin, but not by dialysed iron.

A typical instance of observations leading to the connection between the heating of milk and the production of scurvy is the outbreak of mild infantile scurvy recorded by Hess and Fish in 1914. The infants affected had been fed for several months upon a diet of cows' milk previously heated to 63° C. for 30 minutes.

In 1912 the American Medical Milk Commission had pronounced heated milk equivalent to raw milk for purposes of infant feeding, and the practice of giving orange juice to the infants in the institution in question had thereupon been discontinued.

The symptoms resulting from this diet of heated milk were not those of acute scurvy, but the loss of appetite, failure of growth, and anæmia disappeared rapidly when orange juice or any other antiscorbutic was restored to the diet, or when raw milk was substituted for the pasteurised milk. Similar experiences were recorded by many other observers, and by 1920 we find three vitamins officially recognised and their physiological properties defined:

1. *Vitamin A*—essential not only for growth but for health and vigour at all ages: its

absence leading to weakness or abnormality, not only to tissues of the eye, but also in other parts of the body, especially the lungs.

2. *Vitamin B*—growth-promoting and neuritis-preventing, essential to normal nutrition at all ages. A partial but not complete deficiency leads to impaired growth and a general undermining of health and vigour, with increased susceptibility to infectious disease and interference with normal reproduction.

3. *Vitamin C*—a dietary asset even to a species which shows no scorbutic symptoms, such as the white rat; to be liberally used as a preventive of “latent” scurvy, a condition in which resistance to infectious disease is reduced and in infants, more or less retarded growth, and especially interference with the normal growth and calcification of the teeth result.

In 1920 also Drummond suggested that the “e” of Funk’s original “vitamine” should be dropped, since its original application to the supposed chemical constitution of the accessory substances no longer held good.

(d) **DISCOVERY OF THE ANTIRACHITIC VITAMIN D.**—Rickets, which was formerly so common in England as to be called “Englische Krankheit,” has received many and varied ætiological explanations. In the seventeenth century it was called “rachitis,” because the spine was so frequently involved, and Glisson, in 1650, regarded it as a disease of the bony skeleton due to disturbed nutrition by arterial blood. Later observers recognised that the disease had a close connection with nutrition, but attributed it to the lack of fat, lack of protein, excess of carbohydrate, lack of mineral salts, particularly calcium and phosphorus, etc. (A detailed account of the early history of rickets is given in Hess’s book—*Rickets, Osteo-Malacia, and Tetany*, 1930.) In 1907 another theory—that of unhygienic conditions—was brought forward by Findlay, and by Renton, Robertson, Paton, and Ferguson. They decided from clinical and experimental evidence that the primary factor in rickets was not dietetic, but the result of overcrowding and insanitary conditions, with the possibility of some kind of non-specific bacterial infection as an important factor. In 1914, however, Funk, having in mind the recent discovery of fat-soluble vitamin A, wrote in his work *Vitamins*: “It is very probable that rickets occurs only when certain substances in the diet essential for normal metabolism are lacking, or are supplied in insufficient amount.”

In 1918 Mellanby undertook the study of rickets from the standpoint of vitamin deficiency. As the result of his experimental work (1919, 1920, 1921) upon puppies, he postulated the existence of a fourth (antirachitic) accessory factor, the presence or absence which was the deciding factor in the occurrence of rickets. He found that cod-liver oil was particularly potent in the prevention and cure of experimental rickets, and that beef suet and butter had also a certain antirachitic potency. Olive oil and linseed oil had practically no action, and the vegetable fats as a whole were far less effective than the animal fats. Thus the “antirachitic factor” appeared to have a distribution in many respects similar to that of the fat-soluble vitamin A postulated by McCollum and Davis in 1913, and Mellanby considered it “possibly identical with that substance.”

Mellanby’s work was considered by the Medical Research Committee to place rickets definitely in the category of deficiency diseases, and their Report on Accessory Food Factors (Vitamins) of 1919 included a section which accorded it this position.

Other workers, however, did not accept the identity of the antirachitic vitamin with fat-soluble A. Sherman and Pappenheimer (1920, 1921), for instance, showed that a diet deficient in vitamin A only would not produce rickets, and emphasised the deficiency of phosphorus in their rickets-producing diets.

McCollum, Simmonds, Shipley, and Park (1920) compared the action of butter fat and cod-liver oil, and came to the conclusion that the latter contained in abundance a substance that was antirachitic but which had no specific action on growth or the prevention of kerato-

malacia. McCollum, Simmonds, Becker, and Shipley (1922) proved that the antirachitic substance was distinct from fat-soluble vitamin A.

Moreover, they exposed cod-liver oil to heat and oxidation, a treatment which Hopkins had stated to be destructive to fat-soluble vitamin A, and then found it still antirachitically potent, though it had lost its power to promote growth. This experiment appeared to decide conclusively that vitamin A and the unknown antirachitic agent could not be identical.

Mellanby's work was criticised, and he was blamed for making public claims before his experimental results had been fully published. In a communication delivered by Prof. D. N. Paton to the Glasgow Medico-Chirurgical Society there occurred the statement: "Recently an attempt has been made to prove that it (rickets) is *caused* by the absence of a hypothetical antirachitic factor, probably identical with the fat-soluble A substance. The author of this contention has now abandoned this view, and has reverted to the long-accepted theory that various errors of diet predispose to rickets."

Mellanby (1919) replied to this criticism by a denial that he had abandoned his former views on rickets, insisting that he had made it clear that rickets could not be held to be a deficiency disease of the same nature as scurvy, for the accessory food factor, which played an important part in the ætiology of rickets, was more influenced in its action by other elements of the diet and the environment than appeared to be the case with the antiscorbutic vitamin. Nevertheless, in 1921, he himself summed up the modification of his views as follows:

"The conditions which tend to prevent rickets in puppies are—(1) plenty of calcium and phosphorus in the diet; (2) something associated with certain fats probably identical with fat-soluble A; (3) meat; (4) the possibility of exercise."

On the other hand:

"The conditions which inhibit calcification or increase growth relatively to calcification so that defectively calcified bone results, are—(1) deficiency of calcium and phosphorus; (2) deficiency of fat containing the antirachitic vitamin; (3) excess of bread, other cereals, and carbohydrates; (4) absence of meat; (5) excess of the protein moiety of caseinogen free from calcium; (6) confinement."

Thus Mellanby had so far departed from the original hypothesis that he regarded the causation as due to a complicated series of interdependent factors, but still believed that "probably the most common cause of rickets in children is a combination of relatively deficient antirachitic vitamin and excessive bread."

The pioneer work of Mellanby upon dogs was followed by that of Korenchevsky in 1922 upon rats. This author found that, providing the deficiency were made sufficiently severe—for instance, when the mothers as well as the offspring were deprived of the specific factor—the young developed a condition in all respects similar to rickets.

Goldblatt (1923) extended and confirmed the work of Mellanby and Korenchevsky, showing that deprivation of fat-soluble vitamins alone could produce rickets, though a condition of osteoporosis also was frequently produced. Meanwhile, attention had been directed to another factor in the causation of rickets—lack of sunlight. It had been observed as early as 1890 by Palm that sunlight was very potent in preventing or curing the disease.

In 1919 Huldchinsky reported that rachitic lesions in children healed under the influence of radiations from a mercury-vapour quartz lamp. The action was not local, radiation of one limb healing the rickets of both limbs. No cure resulted, however, if the radiations were filtered through glass; in other words, the ultra-violet rays were the potent agent.

Hess and Unger (1920), Hess, Unger, and Pappenheimer (1921), and McCollum and co-workers (1922) obtained the same results. They found that rats which were exposed to sunlight each day did not develop rickets on a diet lacking in fat-soluble vitamins, while those which were kept in the dark showed definite rachitic lesions.

Hess and Unger (1922) now showed further that other sources of ultra-violet light—the carbon arc and cadmium spark—were efficacious. By means of selective filters they examined different portions of the spectrum, and came to the conclusion that the curative rays were those having a wave-length of $300\text{ }\mu\mu$ or shorter. An interesting experiment was carried out by Goldblatt and Soames (1923) to determine the extent to which rays from a mercury-vapour lamp can supplement a diet deficient in the fat-soluble organic factor. Rats were fed on diets varying in their content of fat-soluble vitamin A from the full quantity to none at all. Half the rats were irradiated for 10 minutes daily, the others, being kept in a warm room with a double plate-glass window, were presumed to receive practically no ultra-violet rays. After 8 weeks the animals were killed, and histological examinations made of their bones, and the calcium content of their femora, tibiae, and fibulae estimated.

The results showed that while the rats on the full quantum of vitamin A grew equally well whether irradiated or not, those on the diets deficient in vitamin A and not receiving irradiation showed signs of rickets or osteoporosis, or both. The irradiated rats on deficient diets showed failure of growth only when the deficiency was as low as 35 per cent. It was concluded that rats fed on a diet containing only 35 per cent. of the minimal amount of A factor to promote normal growth could grow normally and show normal bone calcification if they received irradiation, but that the light rays could not act as a complete substitute for the fat-soluble factor.

The specific action of light on rickets was further shown by experiments by A. Webster and Leonard Hill in 1925. They tried the effect of dried heat and of moist heat on young rats fed on normal diets; when killed they showed no evidence of rickets. They therefore came to the conclusion that, “providing the diet is adequate, conditions simulating the tropics at their worst, but without any ultra-violet light, will not give rise to rickets in young rats.” Other experiments carried out with the same object in view led these observers to consider themselves justified in making the broad generalisation that, provided an animal is supplied with food just sufficient to maintain life and a little growth and a bare minimum of the bone-forming elements calcium and phosphorus, then if ultra-violet light be supplied in adequate amount rickets will not develop however unfavourable the animal’s environment be.

In view of the suggestion that the skin was a depot of antirachitic substance, and that ultra-violet light acted on the skin and stimulated it to pour this into the circulation, they tried feeding animals on the antirachitic diet with portions of skin from freshly killed normal rats. The normal rats were killed and then skinned, as much hair as possible was removed, and weighed portions of the skin were administered daily to the experimental rats. The rats consumed the skin avidly. The results were negative; in fact, the rats that had the 2-gram ration of skin had grosser rickets than the controls.

In an attempt to gain further insight into the mechanism of the action of antirachitic agents, Webster studied the calcium and phosphorus metabolism of these groups of rats in special metabolism cages. Group (1) was fed on the standard rickets-producing diet only. Group (2) had the same diet with the addition of 0.2 c.c. of cod-liver oil per rat per day. Group (3) had no cod-liver oil, but the rats were radiated daily for 10 minutes 2 feet from the mercury-vapour lamp. The experiments lasted the usual period of 4 weeks. During this period the weekly intake of calcium and phosphorus was determined, also the calcium and phosphorus excreted in the urine and faeces. The difference between the intake and combined urinary faecal excretion gave the amount retained.

As a result of their experiments Webster and Hill stated: “These figures strongly support the view that the antirachitic body in cod-liver oil and radiant energy in the form of ultra-violet light both act in the same way in preventing rickets, and that this mode of action is a facilitation of absorption of calcium and phosphorus from the intestine into the blood stream.”

The possibility of an association of fat-soluble vitamin with cholesterol or a similar substance confirmed an observation on the effect of light on cholesterol, made by Ritter in 1901. His accidental observation that specimens of pure cholesterol, prepared 25 years previously by Schulze from gall-stones and lanolin, showed a drop in melting-point of about 30° C. and had become yellow in colour, was afterwards confirmed by Schulze and Winterstein (1904, 1906) and shown to be due to the action of light.

A communication by Rosenheim and Drummond in 1920 shed further light on this association by the further study of certain colour reactions of cod-liver oil and liver fats of other animals. Later, work by Steenbock and co-workers (1924), and Hess and co-workers (1924, 1925), and others showed that it is possible by ultra-violet radiation to induce substances that were previously inert to possess antirachitic properties. Cholesterol when exposed to the influence of ultra-violet radiation was found to be antirachitic; and this result was also obtained when it had been purified to a high degree, and still later Rosenheim and Webster (1926) brought forth evidence to support the view that *ergosterol*, a highly unsaturated sterol, is the direct precursor of vitamin D, and that this substance is transformed into vitamin D when the skin is exposed to direct sunlight. It was concluded, therefore, that sunlight and cod-liver oil cure rickets because sunlight produces in the skin the substance which cod-liver oil provides ready made.

(e) **DISCOVERY OF VITAMIN E.**—The existence of a vitamin essential for reproduction was first suggested by H. M. Evans and K. S. Bishop, of the University of California, in 1922. They found that animals could be kept in good health for periods on synthetic diets containing vitamins A, B, C, and D, and yet seem incapable of producing living young. Fertilisation and implantation of the ova took place apparently normally, but the foetuses died during gestation and were re-absorbed.

In the male, degeneration of the testicle took place, of varying degree of severity, down to a complete and incurable degeneration of all the seminiferous tubules.

In 1923 Sure published a paper in which he supported the contention of Evans and Bishop that a specific food factor existed which was essential for normal reproduction and was distinct from previously described factors: he suggested that this factor should be called vitamin E.

Auderegg and Nelson in 1926 did not consider the evidence for the existence of vitamin E adequate, but Drummond stated in the same year that work had been done in his laboratory which fully confirmed Evans' work as to the existence of vitamin E.

E. V. McCollum and his co-workers (1927) not only accepted the existence of vitamin E, but they also made a suggestion as to its possible function, which seemed at the time a feasible one. They had been troubled for a considerable time by the appearance, among their experimental rats, of an ophthalmia which they felt could not be due to any lack of vitamin A. At one time they attributed the eye trouble to some error in the salt mixture and called it a "salt ophthalmia." In a paper, published in 1927, they correlated the ophthalmia with the death of foetuses on a diet deficient in vitamin E, and regarded both as due to an iron deficiency. They ascribed to vitamin E a function with regard to iron assimilation and stated that, on the diet which they used, the ophthalmia and sterility produced by it could be cured either by adding vitamin E or by substituting ferric citrate for the ferrous sulphate previously used. Later work by these observers (1928) has led them to conclude that vitamin E has no connection with iron metabolism, the "salt ophthalmia" being now regarded as a true vitamin A deficiency.

(f) **DISCOVERY OF THE COMPONENT PARTS OF THE VITAMIN B COMPLEX.**—The work of Goldberger and Wheeler, Lillie and Rogers, begun in 1926, definitely ascribed to a separate factor in the substance hitherto known as water-soluble vitamin B, a connection with the disease known as black tongue in dogs and with pellagra in man. Like the antineuritic factor,

this "P.P." factor was found to be necessary for growth. That such a factor, apart from the antineuritic factor, was necessary for the normal growth of pigeons had already been observed by Cooper (1912). Seidell also (1926) had pointed out the existence of two separate chemical factors in yeast extract, neither of which alone produced growth. Confirmatory evidence of Goldberger's work will be discussed under the heading of "Evidence of the Dual Nature of Vitamin B," but it may be mentioned that for some time confusion as to the nomenclature of the two factors existed. Sherman and Axtmayer (1927) suggested that the antineuritic factor should be described as vitamin F and the antipellagra factor as vitamin G. The Committee on Accessory Food Factors, however, preferred the names vitamin B₁ (thermolabile) and vitamin B₂ (thermostable) respectively.

The existence of a third "pigeon" factor was postulated by Williams and Waterman in 1928. This factor, like the antineuritic factor, thermolabile, is originally present in yeast, and is potent for the growth of pigeons, but not of rats.

A third "rat" factor, labelled vitamin B₃, has been described by Reader (1928), and still another "rat" factor by Coward, Key, and Morgan (1929). These are fully described in the section "Vitamin B Complex."

II. ORIGIN OF VITAMINS.

In investigating the conditions under which vitamins are formed it was at first believed that mammals are unable to synthesise vitamins and are entirely dependent upon the supplies which they obtain either directly from vegetables or at second hand from the flesh or milk of other animals.

The majority of observations tended to support the view that they are formed in the tissues of plants, where they pass into the herbivora, and thus afterwards become available for carnivora, but at different times doubt has been cast upon this view by various workers, and the recent investigations of Bills (1927) and others into the source of vitamin D would seem to show that this vitamin at any rate can be synthesised in the body of animals.

It was believed by Bottomley (1914, 1917) that substances which he called "auximones," and which stimulated the growth of plants, were found as products of decomposition by the action of certain organisms in the soil.

In 1921 Williams and other workers claimed that vitamins, and especially the water-soluble vitamin B, were necessary for the growth of yeast and other micro-organisms. These statements made it very difficult to conceive where and how the vitamin supply of the world was produced.

The experiments of Nelson, Fulmer, and Cessner (1921), however, established the contention that yeast could grow freely without any supply of vitamins, and that it could synthesise vitamin B from a diet of inorganic salt and sugar. They grew yeast for a year on a medium composed of inorganic salts and cane sugar; they made subcultures every other day, and calculated that the final solution only contained $1 \times 50 - 180$ of the original constituents of the medium or yeast. This yeast thus grown on a synthetic diet was found to contain a normal amount of vitamin B. Harden and Zilva (1921) also showed that yeast grown on a pure synthetic diet contained almost as much vitamin B as yeast grown under normal conditions.

(a) **SYNTHESIS OF VITAMIN A.**—The origin of vitamin A was demonstrated by Drummond and Coward during the years 1919–22. These observers showed that dried seeds contained little vitamin A, and that this amount was not increased by germination, but that as soon as the seeds formed green leaves a considerable amount of vitamin A appeared. Experiments made with *Tradescantia* shoots grown on Sach's solution also showed that green plants could synthesise vitamin A from inorganic salts. The production of vitamin A was shown to be

dependent upon the presence of chlorophyll in plants, for etiolated seedlings contained no vitamin A, nor did white leaves from the interior of cabbages. In green seaweeds, containing chlorophyll, vitamin A was present, but red seaweeds contained none. Mushrooms were found to be almost completely deficient in vitamin A. It appears, therefore, that the presence of chlorophyll is essential for the synthesis of vitamin A, and that chlorophyll-containing plants can synthesise vitamin A when fed on a pure synthetic diet.

The association of vitamin A with the pigments, carotinoids, and lipochromes, is fully discussed under the heading of vitamin A, as is also the question of the presence of large stores of vitamin A in cod-liver oil.

It is sufficient to mention the interesting investigations of Drummond (1919–22) into the synthesis of vitamin A by the marine diatom, *Nitzschia closterium*.

This diatom is capable of synthesising the vitamin when grown in sterilised sea-water in the light, and with other similar vitamin-containing vegetable organisms forms the food of a host of minute animals (copepods, amphipods, larval decapods, and mollusca), which in their turn are eaten by many kinds of small fish and other marine animals, salps, squids, and certain molluscs, and these finally are taken by the cod and other fish. In the cod the vitamin is not confined to the liver, but in the period preceding spawning is found in considerable concentration in the roe, both hard and soft, daily doses equivalent to 0.025 gram and 0.05 gram respectively of the fresh soft and hard roe being sufficient to cause resumption of growth in rats.

Other experiments to demonstrate the synthesis of vitamin A have been carried out by Karshan, Krasnow, and Harrow (1927). They pointed out that it is inadequate to compare equal weights of the dried material (ungerminated, germinated, and green) but that an equal number of seeds must be compared. Fifteen rats were kept on a synthetic vitamin A-deficient diet until they became stationary in weight. They were then divided into three groups of five animals. To the diet of Group A was added the equivalent of six seeds of ungerminated corn per rat per day (average weight of seed 0.17 gram); to that of Group B the equivalent of six seeds of germinated corn per rat per day (average weight of seed 0.16 gram); and to that of Group C was added the equivalent of six seeds of green seedlings per rat per day (average weight 0.11 gram).

Within 81 days all the rats in Groups A and B had contracted xerophthalmia and 8 had died. The rats in Group C were in excellent condition and were continuing to gain in weight. Since vitamin A is associated with xerophthalmia these results show that there is a synthesis of vitamin A during the course of the greening.

According to Heller (1928) the amount of vitamin A found in seedlings seems to be a factor depending upon the light rather than upon changes taking place in the process of germination. Some increase is always found in etiolated seedlings. The quantity of vitamin A synthesised is dependent upon the intensity of illumination, length of exposure, and evidently the relative amount of shorter wave-lengths, sunlight causing greatest synthesis, ultra-violet light (3-hour exposure per day) and Mazda light (100 watt, 12 hours per day) being somewhat less efficient. Some synthesis of vitamin A appears to take place even in seeds grown in complete darkness, and the quantity follows closely the rate of growth of the plant.

(b) **SYNTHESIS OF VITAMIN B.**—During the process of germination of seeds, vitamin B, which is located in the seeds, rather than, like vitamin C, in the young shoots and plants, decreases. Heller's experiments indicate that vitamin B is formed not during germination and early growth of the seedlings, but at a later period of development of the plant.

(c) **SYNTHESIS OF VITAMIN C.**—In contrast to vitamin B, vitamin C is produced during the germination of seeds. Heller believes that germination even in the dark produces a considerable amount of vitamin C. The action of light is therefore apparently not essential

to its formation, but since it increases in light-grown seedlings to an extent that is greater than can be accounted for on the basis of the increased growth of the seedlings, its production, like that of vitamin A, is evidently accelerated by increased intensity of light.

(d) **SYNTHESIS OF VITAMIN D.**—After Drummond's explanation of the origin of vitamin A, it was believed that vitamin D must be formed in the same way by ingestion of the marine algæ in which it was originally synthesised.

Doubt was thrown upon the theory by the experiments of Leigh Clare (1927), who found that *Nitzschia closterium*, grown in full sunlight, had of itself no antirachitic potency. Other observers (Voltz and Kirsch (1928), Schittenhelm and Eisler (1928), and others) have carried out experiments which appear to show that vitamin D can be originated in plants during the process of germination without the influence of light, while Bills (1927), Maignon (1927), and Euler and Rydbom (1926) suggest that vitamin D can be actually elaborated in the body without the intervention of light.

(e) **SYNTHESIS OF VITAMIN E.**—According to Evans and Hoagland (1927) vitamin E can be synthesised by plants from inorganic sources. They found that fertility could be restored to rats which had been rendered sterile by a basic ration consisting of casein, starch, lard, milk fat, salts, and yeast, by feeding the Canadian field pea plant, even when the plant was grown from seed in a culture solution of mineral salt. (They admit, however, that they did not avoid bacterial contamination of the nutrient solution.)

III. MODE OF ACTION OF VITAMINS.

Much work has been done in the endeavour to decide how vitamins produce their effect in the body. The first investigators of the conditions produced by the complete absence of vitamins noticed no specific pathological lesions, and believed that the decline in health of the animals was due to a general decline of all its cells. Later experiments, however, have made it clear that the influence of each vitamin is exerted on more or less specific lines.

(a) **THE "HORMONE" THEORY.**—Gowland Hopkins, in a lecture delivered at the University of Edinburgh in 1923, suggested that vitamins might be regarded as "exogenous hormones," confirming his earlier statements to the effect that without the minute quantities of accessory substances, perfectly wholesome and, indeed, from the standpoint of their own functions, absolutely necessary foodstuffs, are not, in any complete and proper sense, utilised at all. They are absorbed, and the energy contained in them is liberated in the body, but the co-ordination of metabolism is absent, and neither material nor energy is employed aright. When the minute addition is supplied these very same foodstuffs become perfectly available as a whole.

On this basis, vitamins are regarded as nutritive essentials not directly concerned with the supply of potential energy, and many other observers have pointed out that the minute quantities required (in the case of a rat the vitamin A requirement is 1/100 milligram daily) are suggestive that their action is more likely to be associated with the repair of protoplasm than with the metabolism of food.

According to Cramer (1920) and co-workers (1921) certain tissues are dependent for their normal action on the continued action of vitamins, which have therefore a positive physiological action corresponding to the action of hormones. The tissues dependent in this way on food "hormones" are mainly, though not exclusively, concerned with digestion, absorption, and assimilation of food. In the case of vitamin A an analogy has been drawn between the atrophy of tissue which occurs both in the intestinal lesions and the typical eye infections and that which occurs in deficiency of certain internal secretions.

From this comparison Cramer (1920) has suggested that vitamin A is a "hormone" with a specific stimulating action, the specificity of its action being further confirmed by the

rapidity with which the intestinal or ocular lesions recover when vitamin A is administered after a previous deficiency.

Similarly, in the absence of vitamin B, the normal assimilation of the food is impaired owing to the atrophy of the lymphoid tissues, which takes place not only in the intestine but throughout the body, and is reflected in the circulating blood by a lymphopenia.

The avitaminous condition of the lymphoid tissue is practically identical with that produced by exposure to X-rays and radium. These agents, if given in appropriate dosage, not only have the same effects as regards the lymphoid tissue, but they also produce the same general effect on the animals—they lose weight and die in a state of emaciation. Incidentally it may be mentioned that in this condition there are also degenerative changes in the seminiferous tubules of the testes similar to those observed in avitaminosis. The suggestion that the effect produced on the general nutritive condition of the animal by these different agencies may be due to their selective action on the lymphoid tissue finds confirmation in the following facts. (1) It is known that lymphocytes are concerned in the absorption and assimilation of fats, and Cramer has presented experimental evidence to show that the assimilation of protein from the intestinal canal is brought about partly by the agency of leucocytes. (2) According to D. H. Patterson (1921), in certain marasmic conditions in children, not due to interference with the food supply, there is an atrophy of the thymus and of the Peyer's patches, and no other lesion has so far been found to account for the condition. (3) Atrophy of the spleen and an almost complete disappearance of the thymus occurring in pigeons, both in inanition and in experimental beri-beri, were reported by Weiske in 1896, and by Findlay in 1921. (4) In a paper published by Drew, Mottram, and Cramer, in 1921, it was pointed out that human pathology furnishes evidence that an important function of the lymphoid tissue is to maintain the nutrition of the body—a view which is also held by the French physiologist—Jolly, on similar grounds. (5) An interesting confirmation of this view was adduced by Carrel and Ebeling (1923), who found that fibroblasts cultivated *in vitro* could only grow in serum when lymphocytes were added to it, and who argued from this that the lymphocytes synthesise from the serum substances necessary for growth. (6) There is evidence also that the organism recovers more rapidly from a lymphopenia induced by radiation when an abundant supply of vitamin B is administered. (7) The absorption of fat has been shown by Mottram, Cramer, and Drew (1922) to be greatly stimulated by the presence of vitamins. (8) The secretion of the pancreas has been shown by Voegtlin (1919) and by Anrep and Drummond (1920) to be increased by extracts of foods rich in vitamins. (9) Appetite and food consumption have been shown by Drummond to receive their essential stimulation from vitamin B. All these pieces of evidence would appear to point to the conclusion that vitamins do act as stimulators of cell activity as a whole. Cramer, Drew, and Mottram (1921) have added the qualifying suggestion that vitamins, while essential for the maintenance of life of a highly differentiated animal as a whole, are not essential, necessarily, or probably, for the life of its individual cells. They base their supposition on the fact that certain living cells, such as those found in hypertrophy of the adrenals, in Lieberkühn's follicles, undergoing mitosis, and in transplanted tumours, do continue to grow actively in the absence of vitamins.

(b) **ACCELERATION OF TISSUE OXIDATIONS.**—That the action of vitamins might depend upon their power to accelerate oxidation in the tissues was suggested by Freudenberg and György (1920). They found that surviving liver cells and cells from the jejunal mucosa had their oxygen demand increased in the presence of vitamin rich extracts. Other observers, including Aberhalden (1920), Anderson and Kulp (1922), Caridroit (1922), etc., had come to a fairly general agreement that deprivation of vitamin B led to a reduction of the oxygen utilisation of the whole animal. An investigation of the whole question by Drummond and Marrian (1926), however, led them to conclude that the oxidative activity of the tissues remain unim-

paired by deficiency of vitamin B until the final phase of malnutrition, and that the real interference takes place in the progressive failure of appetite due to its lack of essential stimulation by the presence of vitamin B.

(c) **MAINTENANCE OF MINERAL EQUILIBRIUM.**—The work of Mazé (1927) on mineral nutrition has already been mentioned. He believes that vitamins supply the organic mineral which is necessary so that synthesis of the inorganic elements can proceed in the body, *i.e.* that in the presence of a minimal amount of vitamin the organism can manufacture a further supply.

A rather different view is that of Kollath (1926), who considers that avitaminosis in general may be due to a disturbed condition of phosphatide and mineral. He draws a parallel between vitamin activity and the growth of the influenza bacillus on agar containing potassium ferro-cyanide, which has been previously irradiated with ultra-violet light. He suggests that just as two factors produced by the irradiation (which he designates the X factor, containing iron, and the V factor, probably a lipoid) are effective only in their reactions one upon the other, so the phosphatide and mineral in the living cell are in equilibrium when there is no vitamin deficiency. He considers the V factor vitamin-like in nature and has found it to be present in the water-soluble phosphatide preparation from living plant material. This extract, when added to diphtheria or influenza bacilli, increased growth. Ordinary lecithin alone, or in combination with the X factor, did not promote growth. The phosphatide preparation contained no vitamin A, C, or D, but delayed, or temporarily improved, symptoms of beri-beri in pigeons on a vitamin B-free diet. The administration of the phosphatide extract was followed by a fall in blood sugar. Also in dogs the administration of the phosphatide preparation either by mouth or by injection caused a fall in blood sugar. Feeding lecithin had no effect in alleviating the symptoms of beri-beri, but an injection of lecithin delayed the appearance of these symptoms. The phosphatide preparation was more effective than lecithin in producing growth in mice on a basal diet of oats, bread, and water. Kollath believes this to be due to the phosphatide in the cell being in combination with other important factors. This combination is destroyed in the usual preparation of lecithin, which thus becomes biologically denatured.

A relationship between vitamins and iodine has been suggested by Harvey (1928). He observed that if cod-liver oil (which contains iodine) were given to goats it caused more iodine to pass into the milk than if the goats were fed on equivalent amount of potassium iodide and olive oil. From this he deduced that the specific constituents of cod-liver oil might have some effect on iodine metabolism. He employed a preparation consisting of iodised jecoleic acid incorporated with a vitamin concentrate from the oil in cases of Graves' disease, with good results. When the iodised fatty acids or the vitamin fraction were given alone the effect was less successful; it is suggested, therefore, that combination of both portions is necessary for the maximum effect. The question thus arises of a positive vitamin effect in contrast to the negative action in deficiency diseases.

(d) **REGULATION OF "TROPIC" CHANGES.**—Still another theory as to the physiological rôle of vitamins is that expressed by Ganassini and Mancini in 1920, that they regulate trophic changes in synergic connection with the trophic regulatory function of the glands of internal secretion. In the experience of Cascella (1927) also, drawn from his investigations on children treated with preparations of isolated vitamins, the action of vitamins is controlled and modified by the endocrine glands. He considers that vitamins act as catalysing agents which display activity and are subsequently eliminated.

IV. VITAMIN BALANCE.

The distribution of vitamins in natural foodstuffs is such that with a varied diet a sufficient quantity of all is likely to be present. The question of a definite proportionality seems to be

rather one of natural selection than a quantitative dosage of separate vitamins. It is necessary, however, that the selection of foodstuffs should be controlled by a knowledge of the action, separate and combined, of the vitamins in question. An imbalance of vitamins may have definite toxic effects, just as surely as a partial deficiency of all, or a complete absence of one.

(a) **EFFECT OF VITAMIN BALANCE ON GROWTH AND METABOLISM.**—An inhibiting effect of an incorrect proportion of vitamins A and B in the diet was shown by Hopkins (1923). Vitamin A and B were supplied to rats on a synthetic dietary. In one experiment each was supplied in very small quantity, but the amount of the one bore a definite ratio to the amount of the other; growth was maintained, but was subnormal. In a second experiment the absolute amounts were increased, but the ratio was undisturbed; growth was normal. Next, both vitamins were given in relatively very large amounts, but with the ratio still the same; growth was again normal and the animal's health was undisturbed. When, however, in another case, animals received A in the small amount employed in the first experiment and at the same time took B in the large amount of the third experiment, they showed little or no growth and failed to flourish, though there were no acute symptoms to suggest a direct deleterious effect from the large dose.

A theory to explain the inhibiting action on growth of an imbalance of vitamins A and B has been advanced by Richardson, Palmer, and Kennedy (1928). On a ration containing 18 per cent. purified casein, sufficient salts and energy-yielding material, with 5 per cent. butter fat and 0.6 gram pure yeast daily, rats failed to grow at the normal rate, remained sexually immature and often developed greasy skin and fur. Butter fat seemed to possess some quality that was depressing to growth and related to the greasy condition, for 15 per cent. lard or olive oil gave better growth and health than 15 per cent. butter fat. The ratio of protein to fat bore no relation to the problem. The hypothesis is advanced that in highly purified rations some compound is lacking which links vitamin A with the fat. Thus the vitamin, which is naturally in a combined condition, is free and might be antagonistic to vitamin B. It might also cause a faulty utilisation of fat.

(b) **EFFECT OF VITAMIN BALANCE ON RESISTANCE TO INFECTION.**—Following Cramer's suggestion that the whole vitamin activity is dependent on the intestinal wall changes, producing a secondarily deficient or altered absorption and an increased local susceptibility to infection, experiments have been carried out to discover the effect on such intestinal permeability of the vitamin ratio of the diet.

According to Agnes H. Grant (1926) a severe disturbance in the proper ratio between vitamins C and D in the diet of guinea-pigs decreased the normal resistance of the intestine to migration of intestinal bacteria (*Bacillus ærtrycke*) into the blood stream, whether C was in excess and D deficient or the reverse. Oxidised cod-liver oil (vitamin A destroyed) behaved likewise. Oxidising orange juice destroyed its power of preventing decreased intestinal resistance due to the cod-liver oil factor. Excess of Ca seemed to favour migration of bacteria into the blood stream and rapid multiplication in spleen and lungs when both C and D were deficient, and when given with an excess of vitamin D and a deficiency of vitamin C, as many as 75 per cent. of the guinea-pigs studied have been infected. With these unbalanced diets the infections were found in animals inoculated by mouth with cultures of *Bacillus ærtrycke*, and also in uninoculated animals. Only slight evidence was apparent that the resistance of the intestinal wall was greater for the organisms normally present than for those introduced in pure cultures. Intestinal ulcers were produced with a deficiency of vitamin C and developed more rapidly and showed less inclination to heal when the diet contained an excess of cod-liver oil.

Grant further suggests (1930) that there is a definite relationship between Ca, vitamin C, and vitamin D in normal diets. Vitamin C influences the course of tuberculosis to such an

extent that the disease cannot be successfully treated unless there is an abundance of this factor in the diet. Further experiments were made in the rat to determine how far the lowered resistance produced by deficiencies and excesses of Ca and vitamin D in the maternal diet could be modified by rebalancing Ca and vitamins C and D. It was first shown that a prolonged excess of vitamin D in the diet (over two or three generations), either with a normal or deficient amount of Ca, hastens the spread of tuberculosis throughout the lungs even when the excess vitamin D is discontinued after inoculation. Substitution of vitamin C for vitamin D at the time of inoculation or the addition of vitamin C to a vitamin D-deficient diet appeared to increase the amount of tuberculosis, but the addition of vitamin C (orange or tomato juice) to a diet containing vitamin D increased the resistance. The diet given before inoculation, or the resistance of the rats when weaned, is far more important than their weight at this time in determining the degree of tuberculosis which will develop in the lungs. The offspring of mothers with prolonged excesses or deficiencies in their diet often develop extensive lesions on diets that fully protect normal young of the same weight when weaned.

(c) **TISSUE IMBALANCE.**—Of the relation between the vitamins contained in foodstuffs and the vitamins contained in the tissue of the animal organism little is known. An interesting suggestion for cancer research is contained in the work of Burrows, Jorstad, and Ernst (1926) in this connection. While imbalance of vitamins in the food may have a certain effect in lowering the resistance of the tissues to infection, it is possible that a corresponding imbalance might be present in the tissues themselves, and produce still further ill-effects. Such a tissue imbalance of vitamins A and B has been stated by Burrows and Jorstad and Ernst to be present in cancerous tissues.

(d) **MUTUAL REINFORCEMENT OF VITAMIN ACTION.**—That the various vitamins are able reciprocally to reinforce each other's action has been indicated by several groups of observations.

The growth-promoting effect of vitamin A is stated by Sumi (1929) to be increased by supplementing it by vitamin D (irradiated ergosterol).

Euler and Widdell (1925) have indicated a co-action of vitamins C and D by experiments which show that lemon juice favours the activity of the antirachitic vitamin, and that a large excess of lemon juice prevents the sudden loss of growth stimulation which occurs after 8 weeks when a large excess of cod-liver oil is given.

Grant (1930) believes also that vitamin C plays some part in the development of rickets, for she has found that rickets is cured more completely when both vitamins C and D are added than with vitamin D alone.

In pneumococcal infection simultaneous administration of vitamins B and D has been shown by Eicholz and Kreitmar (1928) to cause a greater reduction in mortality than the administration of vitamin D alone.

Pfannenstiel found these results in full accord with his own in regard to the increase in the bactericidal power of the serum of healthy full-grown rabbits. When these received, for a period of 6 weeks, an addition of cod-liver oil to their normal diet, and afterwards an addition of yeast, their serum at the end of the feeding period showed a pronounced increase in its power to kill typhoid bacilli, an increase which was of notably long duration. From the addition of yeast, cod-liver oil, or lemon juice alone to the diet, on the other hand, no noteworthy increase in bactericidal power could be observed in the serum.

In experiments on rabbits with tuberculosis, Pfannenstiel and Scharlau (1930) did not note any improvement following the administration of vitamins A, B, and D singly. By the simultaneous peroral administration of vitamin B (yeast) and vitamin D (viosterol), however, it was possible not only to increase the bactericidal power of the blood serum but to influence the tuberculosis favourably. Rabbits receiving both vitamins did not develop tuberculosis

following a weak infection, and other rabbits with severe tuberculosis showed a marked tendency to recover when given both yeast and viosterol. The therapeutic action of vitamins B and D was not increased by the additional administration of vitamin A (cod-liver oil).

(e) **HYPERVITAMINOSIS.**—The question of the toxic effects of one vitamin has received particular attention since the discovery of the ill-effects of a comparatively small dosage of irradiated ergosterol.

It was suggested by Takahashi as early as 1922 that massive doses (16 mgrm.) of his "biosterin" (a concentrated preparation of vitamin A) proved fatal to rats. With the contradiction by Drummond and co-workers of Takahashi's claim to have isolated vitamin A, the suggestion was made that the effects he had observed were due to poisonous by-products in his preparation, and no further attention was paid to the question of a vitamin excess.

In 1926, however, arose the controversy between Agduhr and Höjer upon the toxic effects of cod-liver oil. Agduhr (1925) reported that feeding excessive amounts of cod-liver oil to various animals produces degenerative changes in the heart, with pigment atrophy, vacuolous and waxy degeneration, and transformation of muscle into connective tissue cells, thinning of the cardiac walls, and dilation of the lumina of atrium and ventricle.

In spite of Höjer's contention (1926–27) that these changes only occurred when vitamins B and C were deficient in the diet, Agduhr maintained that they were due to the maximum doses of cod-liver oil, and quoted Takahashi's experience with biosterin. At this time the toxic effect was believed to be due to the vitamin A content of the cod-liver oil, not, as is now known, to its vitamin D content.

A hypervitaminosis A arising from the use of biosterin has also been described by Munehisa (1929), but its occurrence was not very definite, since the controls displayed similar reactions in some cases. He states, however, that rabbits receiving excess amounts of vitamin A showed a decreased excretion of urine, an increase in body weight, muscular atrophy and hind limb paralysis, and died within 7 to 10 days. They retained a good appetite until the end. The total combined excretion of Ca, P, and Mg increased in both groups beyond the normal figure, but the increase was much more noteworthy in the biosterin group.

The question of hypervitaminosis D is so extensive that it will be best considered in detail under the heading of vitamin D. (See Section XXXIII, p. 173.)

It may be mentioned here, however, that the influence of other vitamins on this particular toxicity has received a good deal of attention.

In 1923 Hopkins drew attention to the fact that excess of cod-liver oil had often been found injurious to rats unless "balanced" by an increased vitamin B allowance.

On somewhat similar lines Euler and Widdell (1925) stated that excess of cod-liver oil caused disturbances in the growth and bone formation of rats unless balanced by vitamin C.

More recent investigations by Harris and Moore (1928, 1929) seem to point to an imbalance of vitamin B as one of the causes of the toxic effects of vitamin A and D concentrates. They found that excess of vitamin D in the diet, leading to the typical lesions of hypervitaminosis D, had a lessened toxicity when vitamins B and C were added in large quantities.

They found also (1929) that rats receiving massive doses of vitamins A and D concentrate from cod-liver oil, in conjunction with a diet deficient in the vitamin B complex, developed loss of hair and severe skin lesions symptoms resembling B avitaminosis.

V. NATURE OF VITAMINS.

In tracing the discovery of vitamins from the original conception of their connection with some morbid condition it has been seen that they could be studied only by the effects of their absence. For many years their investigation was approached more closely from the biological than from the chemical or physical standpoint, but there has been a tendency during recent

years to attempt to resolve each vitamin to its exact chemical formula. Although many workers have succeeded in concentrating them in a very high degree of purity, and in proving beyond doubt their prophylactic and curative connection with the morbid condition for which each is responsible, their ultimate nature remains still in doubt. Yet, to take only one example, the proved curative effect on rickets of irradiated ergosterol, in a dosage of 1/20,000 mgrm. per day, would seem to show that these "elusive and mystic bodies" of which Oliver (1929) makes the somewhat sweeping statement that we "know practically nothing," must soon yield their ultimate problem to the efforts of chemical research.

(a) **SOME THEORIES OF THE NATURE OF VITAMINS**—(1) **Physiological Stimulants ("Hormones")**.—In 1911, when Hopkins first described his experiments on the effects of absence of "accessory food substances," the question arose as to whether vitamins were actually indispensable structural tissue units, or whether they were to be regarded as essentially dynamic and catalytic. The result of numerous discussions was the almost universal conclusion that "the function of accessory food substances must be that of stimulants rather than structural units."

This conception has been amplified by workers, such as Mazé (1927) and Vogt (1927), who are inclined to regard vitamins as the hormones of the vegetable world. Mazé, working on the relation of vitamins to the mineral nutrition of the living cell, has suggested that they consist of highly unstable mineral organic compounds whose presence in plants is necessary in a constant definite ratio before normal metabolism can proceed. Vogt (1927) has also attempted to demonstrate an intimate relationship between hormones and vitamins, and Evans and Burr (1927) draw attention to the interesting resemblances between the properties of ovarian hormone and vitamins A and E.

(2) **Inorganic Chemical Substances**.—The numerous efforts to isolate vitamins from the substances known to contain them will be described in detail in the chapters dealing with each separate vitamin. These efforts date from Funk's idea (1912) that "vitamine B" was a substance of the nature of a pyrimidine base.

A similar theory was advanced by Williams and Seidell (1916) when they tried to show that vitamins were tautomeric substances existing in active and inactive form. They suggested that "vitamine" was a pseudobetain, and Williams showed that alpha-hydroxyl-pyridine possessed antineuritic power which was lost on standing.

Dubin and Lewi (1921) considered the essential element of a vitamin to consist of a phytin molecule, which is a double calcium magnesium salt of inosite pentaphosphoric acid. A tentative analysis of a product prepared from corn, autolysed yeast, and orange juice materials, containing therefore vitamins A, B, and C, showed the following chief constituents. Calcium (CaO), 10 per cent.; phosphorus, 15 per cent.; nitrogen, 3.5 per cent.; fat, 2.5 per cent.; iron, 0.3 per cent.; silicates, 5.6 per cent.; moisture, 10 per cent.; the remainder being made up of the rest of the phytin molecule.

The presence of carbon, hydrogen, oxygen, and sulphur, and the absence of nitrogen, phosphorus, and the halogens was inferred in the constitution of a highly concentrated extract of both antirachitic and anti-ophthalmic vitamins prepared by Dubin and Funk (1924). This observation was confirmed by Drummond, Rosenheim, and Coward (1925), and Drummond and co-workers have greatly extended this line of investigation in their attempts to concentrate still further the active fraction of cod-liver oil.

In the case of vitamin C extremely concentrated fractions prepared by Zilva (1926) have been found to contain very little nitrogen but traces of iron phosphorus and sulphur. Since Zilva has succeeded in differentiating vitamin C into two factors—the "antiscorbutic" and the "reducing"—he has been led to speculate on the actual size of the active molecule, which he thinks may be somewhat larger than that of a hexose.

Vitamin D is almost universally believed to be the direct product of irradiation or ergo-

sterol, which is, according to Rosenheim and Webster (1926), a sterol of a definite chemical structure. Only a sterol of an unsaturated and labile type, possessing three double bonds and a hydroxyl radical, was found to possess the faculty of being changed into a substance of high antirachitic potency. This product of the irradiation of ergosterol is considered by Rosenheim and Adam (1929) to be probably of a ketonic nature.

Vitamin E, in the most highly concentrated fraction of wheat-germ oil, is stated by Evans and Burr (1927) to resemble very closely in chemical characteristics the most concentrated form of vitamin A prepared by Drummond. They remark, however, that "the best fractions are still too conglomerate for trustworthy speculation as to the true nature of the vitamin."

(3) **Organic Chemical Substances.**—Since Steenbock in 1919 advanced a theory that vitamin A might be a yellow pigment, or a substance closely allied to the pigments found in egg-yolk, yellow corn, etc., many investigations have been conducted on these lines. Special attention has been directed to the close association between vitamin A and carotin, a substance which is widely distributed in both the animal and the vegetable kingdom, and which has been proved capable of curing experimentally produced avitaminosis A.

Euler and co-workers (1928, 1929) have indeed suggested that the vitamin A of vegetables is a carotin and differs from the vitamin A found in animal oils, while Moore (1929) considers it probable that carotin acts as a pro-vitamin.

So late as 1929, however, Collison, Hume, and Smedley-Maclean's investigations into the discrepancies of the results of other workers have led them to state that "there is at present no evidence available finally to decide the relationship between carotin and the substance responsible for the biological activity of vitamin A in liver extracts."

The frequent association of vitamin A with the lipochromes in various tissues has also originated a theory that the vitamin might be an actual member of the lipochrome class of pigments. The observations of Steenbock (1919), however, and of Drummond and Coward (1920) on this subject have led to the conclusion that the more or less constant relationship of the two substances is to be regarded as accidental.

(4) **"High Energy Compounds."**—Professor Baly (1925) has enunciated a theory that vitamins are not specific chemical substances but compounds in a high state of chemical activation. He believes that food values depend not only on their content of calories but on their energy content, and that the remarkable properties of vitamins are due to the fact that they have a very high energy content. He expresses his conception of a vitamin as follows: "A normal healthy tissue may be shown by biochemical methods to contain one or more vitamins; if this same tissue loses its excess of energy, then all the tests for the presence of vitamins will fail, but the chemical composition of the tissue will in no way have changed." He observes that this explanation of their nature is conformable with what is known of their chemical and physical properties. Their differentiation by solubility and volatility, for example, is quite in keeping with the idea of there being two phases of the same compound with different energy contents.

VI. THE STABILITY OF VITAMINS.

A knowledge of the conditions affecting the stability of vitamins is of importance from two points of view:

(a) **THE COMMERCIAL AND DOMESTIC TREATMENT OF FOODSTUFFS CONTAINING VITAMINS.**—With the necessity imposed by modern conditions of existence, for depending to a certain extent upon foodstuffs prepared and preserved by commercial methods, the question has arisen of modifying these methods in such a way that the foodstuffs shall not lose their vitamin content. If food could be provided in bulk in its natural state, and if the civilised palate could be persuaded to content itself with a diet almost entirely uncooked, the problem of vitamin

deficiency would be practically non-existent. Economic factors and difficulties of transport, however, make storage and preservation a necessity, while not only palatability but digestibility and freedom from bacterial contamination set a limit to the proportion of the daily diet which can be eaten raw. Some of the most interesting facts which have emerged from the examination of foodstuffs for their vitamin content are those which relate to the effect of the commercial process of canning. In America particularly this process has reached a very high state of efficiency, and the researches of Eddy, Kohman, Sherman, and many others from 1926 onwards seem to show that it is possible to preserve for human use the most perishable foodstuffs without destroying their vitamin content. From the biological point of view, consideration of the destruction of vitamins opens an almost limitless field of investigation. It has been observed in the previous section that vitamins can be studied physiologically only by the effects of their absence. The partial deficiency of vitamins, however, is a very important aspect of this subject. Experimental animals have been found to show a wide variation in the symptoms evidenced according to the degree of deficiency. Peters' happy phrase, "the Ministers of Metabolic Change," is a sufficient indication of the supreme importance of an adequate supply of vitamins to the human body. If the vitamins are essential factors in the maintenance of the life of the cell itself, it needs no great power of vision to realise that the cell must have its full quota of this vital necessity. Clinical experience has borne out scientific evidence on this point from all sides, and more and more clear becomes the realisation that commercial progress must keep step with biological investigation if humanity is not to be deprived of these substances which are necessary for the maintenance of health as well as life.

(b) **THE INVESTIGATION OF THE CHEMICAL NATURE OF VITAMINS.**—Every attempt to concentrate the vitamin element in a natural foodstuff is attended with the difficulty of destroying it by the processes involved. Heat, oxidation, treatment with acids or alkalies, etc., are all manipulations to which the different vitamins exhibit a varying susceptibility.

The special properties of each vitamin with regard to destructive agencies will be described under the separate heading for each.

VII. VITAMIN MINERAL RELATIONSHIPS.

Experiments on the relationship between the various mineral salts and vitamins shows them to be more closely connected than was at first recognised.

(a) **COMPLETE ABSENCE OF MINERAL SALTS.**—The complete absence of mineral salts has been found by Kauffman-Cosla and Roche (1927) to produce a set of symptoms which they have called "dysoxidative carbonuria," which are also produced by avitaminosis. They found that an animal receiving a vitamin-deficient diet with an excessive calorie value showed an elimination of imperfectly oxidised carbon in the urine, and that the absence of mineral salts, particularly Ca and P, produced the same result.

(b) **CALCIUM, PHOSPHORUS, AND MAGNESIUM RELATIONSHIPS.**—The relationship between Ca, P, and Mg and vitamins have been investigated by Haag and Palmer (1928), who, dealing with the effect on rats of variation in the proportions of Ca, Mg, and P contained in rations, intended to be reasonably adequate in other respects, conclude that a more or less balanced condition of Ca, Mg, and P salts of the ration is essential to normal growth and functioning. High Mg as a disturbing factor in nutrition is definitely indicated by the data on growth and mineral balance, the extent of this disturbance being conditioned by the intake of Ca, P, and vitamins (or their carriers). An increased requirement of vitamins (in the form of cod-liver oil and yeast) was found in groups with a high magnesium content. Calcium or phosphorus deficiency, or both, can give rise to nervous symptoms closely resembling the polyneuritis of vitamin B lack, and paralysis, convulsions, tetany, and other nervous symptoms may all

arise. It is suggested by Corlette that such diseases as osteoporosis of horses (bran disease), milk fever, and lactational dyspepsia of cows, epizootic stringhalt, geophagia (earth-eating), "sand disease," of horses, coprophagia, some cases of "forage poisoning," convulsive "ergotism," lathyrism in man and animals, scrapie of sheep, laminitis of horses, and bone chewing, may all have a common ætiology in a calcium, or calcium and vitamin D lack.

It has been suggested by Delbet and Palios (1929) that the effect of magnesium is to aid the animal to synthesise its own vitamins. They state that animals on diets deficient in vitamins C and D respectively survived slightly longer when $MgCl_2$ was added than without it.

(c) **SODIUM AND POTASSIUM RELATIONSHIPS.**—The necessity of sodium and potassium in a diet containing full quantity of vitamins and other mineral salts has been proved by experiments carried out by J. L. St. John, 1928, who has further investigated the amount of sodium required. Rats fed on rations containing sodium varying from 0.02 to 0.5 per cent. failed to maintain successful growth when the sodium was 0.30 per cent. or less. The animals receiving smaller amounts of sodium were seriously affected. There was no reproduction on any of the vitamins used. Vitamins A, B, and D were adequate, so that poor growth is not attributed to vitamin deficiency. Immediate improvement resulted when the stock rations were restored.

(d) **MANGANESE RELATIONSHIP.**—Experiments carried out by McCarrison (1927) primarily with a view to ascertaining whether the high nutritive value of wheat could be correlated with its high manganese content show that the presence of manganese in low concentration (0.0327 mgrm. daily, representing a concentration of one part of manganese in 617,700 parts of the food eaten) in a full vitamin diet exercises a favourable influence on growth.

It is significant that manganese occurs most abundantly in those parts of plants in which vitamins are also most abundant. Thus, cabbage leaves contain as much as 0.64 mgrm. per 100 grms. of the fresh material; turnip leaves, 0.21 mgrm.; asparagus tops, 0.19 mgrm.; cress, 0.16 mgrm.; leek, garlic, and onion from 0.05 to 0.09 mgrm.; while fruits—orange, lemon, strawberry, grape—contain in ascending order from 0.1 to 0.73 mgrm. of manganese per 100 grms. of the fresh material. Whole wheat is particularly rich in manganese; while paddy is poor in it. It appears to be concentrated in the outer layers of the wheat grain; Bertrand and Rosenblatt (1921) having found as much as 3.9 mgrms. in each 100 grms. of dry wheat bran, while estimations made by Dr. R. V. Norris have shown the whole grain to contain 4.82 mgrms. per 100 grms. of the dry material. Like vitamin B it is, therefore, largely removed in the process of manufacture of white flour.

McCarrison (1929) has recently attempted to trace a connection between the manganese-vitamin relation and the production of a variety of lymphadenoid goitre, unrelated in its origin to vitamin deficiency. Rats were fed on different diets, all of which were deficient in both manganese and vitamins. Lymphadenoid goitre, similar to that described by Williamson and Pearse in 1929, was produced in these animals, and McCarrison believes that this disease is not due to deficiency of iodine in the food, but to an insufficiency of vitamins of the A, B, C, and D classes associated with a deficiency of certain inorganic elements, of which manganese appears to be one. In man it may be expected to occur in young and growing persons who subsist on diets composed largely of white flour poor in manganese and vitamins or other vitamin-poor carbohydrates, protein, and fats, with a paucity in, or absence from, the diet of fresh fruits and green leafy vegetables. Such diets, judging from their effects on rats, induce in the thyroid gland a state of physiological subnormality which is the basis of lymphadenoid goitre.

(e) **IRON AND COPPER RELATIONSHIP.**—Similar relationships between iron salts and vitamins are suggested by nutritional experiments carried out by Waddell, Elvehjem, Steenbock, and Hart (1928) for the purpose of investigating anæmia in young rats. The effects of inorganic iron salts were studied, making use of a commercial chloride and a C.P. ferrous

ammonium sulphate. The iron salts were fed at a level of 2 mgrm. of iron daily, 6 days a week, and later at a level of 0.1, 0.15, 0.5, and 1.0 mgrm. In no case did these small additions of iron correct the anæmic condition, and all the animals died after showing only slightly increased hæmoglobin. The effect of iron in the form of ash and dried lettuce was also tried. Dried lettuce, in a dosage of 19 mgrms. daily, 6 days a week, was found effective in correcting anæmia. Beef liver, kidney, and muscle, yellow corn, and wheat were selected for another experiment. The quantities of these animal tissues were sliced, dried, and ground, and the corn and wheat were finely ground. All materials were fed at levels of 0.5 gram per rat 6 days per week. This level in the case of beef liver introduced about 0.15 gram of iron. Beef liver resulted in steadily increasing hæmoglobin formation as well as good growth and physical appearance. Beef kidney was less favourable and beef muscle practically inert. Corn and wheat were less valuable and in most cases the animals died. Further experiments were initiated to determine reasons for the effectiveness of liver material. Various salts of iron, chloride, sulphate, acetate, citrate, and phosphate were prepared. These salts were fed at a level of 0.5 mgrm. 6 days per week. All these salts failed to produce any material increase in hæmoglobin, the majority of the animals dying in 4 to 8 weeks. Hydrochloric acid extracts of the ash of lettuce, yellow corn, and beef liver were made. These were compared in feeding experiments with ferric chloride and with the ash of liver ignited with calcium carbonate and were fed in such amounts as to furnish practically 0.5 mgrm. of iron. Marked hæmoglobin regeneration resulted in all cases except with the addition of ferric chloride alone. It is concluded that in addition to the iron, which is essential, the ashes and extracts of the ashes of corn, lettuce, and beef liver contain some other substance necessary to restore hæmoglobin, evidently inorganic in nature.

An investigation into the presence of copper as a supplement to iron for hæmoglobin building, and the probable manner to which it functions, leads to the suggestion that it may act as a catalyser for certain reactions.

The copper was contained in the hydrogen sulphide fraction of the ash of a liver preparation which had proved effective in the treatment of pernicious anæmia, and was found most potent at levels of 0.05 and 0.10 mgrm., with 0.05 mgrm. of iron as ferrous chloride in addition.

The relation of iron metabolism to the presence of vitamin E was at one time believed by Simmonds, Becker, and McCollum (1927) to be specific, in the same way that phosphorus and calcium metabolism are specifically related to vitamin D. Their discovery that ferrous sulphate in the diet produced a special "salt ophthalmia," and at the same time a decrease in fertility, both of which yielded to the administration of vitamin E, led them to believe that simultaneous deprivation of iron and vitamin E had a more potent toxic effect than deficiency of vitamin E alone.

Their views were later shown to be untenable by the studies of Mattill (1927) on the oxidative destruction of vitamin E in fats and Mason on the iron content of the blood. These studies will be discussed more fully under the heading of vitamin E.

An interesting connection between iron and vitamin D has been suggested by Waltner (1929). He observes that an amount of reduced iron which, with a complete diet, has no effect on the blood, produces a marked anæmia if the diet is a rickets-producing one. Other toxic effects of a large inclusion of iron in the diet (2 per cent.), such as retardation of growth, injury to lactation, and reduction of fertility, he states can be counteracted by the addition of vitamin D.

VIII. VITAMINS AND NUTRITION.

(a) **EFFECT ON NORMAL GROWTH.**—Although vitamins have been studied to a very great extent from the point of view of deficiency diseases and their treatment, they have also a

purely physiological importance. As Hess has truly observed: The harmful effects of food deficiencies should not be associated in our minds essentially or chiefly with specific diseases such as scurvy or rickets, but rather as disorders of nutrition, producing slight and manifold disturbances of function."

Not the least important factor in the establishment of normal growth is the presence of an adequate supply of vitamins, particularly in regard to mineral metabolism, and, therefore, in the development of the skeleton. It has been stated by Funk that in the lower animals delayed growth may be remedied in later life by the addition of the accessory food factors, but that in man there is probably no response once the growth period is passed. He believed that some cases of stunted growth might arise from such dietetic deficiencies.

Cramer's experiments (1922) on "vitamin underfeeding" during long periods tend to support the belief that animals subjected to vitamin deficiency for the first few weeks after birth receive permanent damage as the result of their unfavourable dietetic conditions, and are not able to respond to an increased vitamin supply by increased growth. This effect was intensified if the stock from which young rats were derived had also been subjected to vitamin-underfeeding, while conversely the growth of rats which came from a vitamin-rich stock and which since birth had received an ample supply of vitamins was not impaired by subsequent underfeeding of vitamins.

(b) **EFFECT OF DEFICIENCY DURING PREGNANCY AND LACTATION.**—Improvement in the stock as indicated by more rapid growth by addition of vitamins to the diet was particularly noticeable during pregnancy and lactation.

The mother herself always lost weight during pregnancy and lactation on the vitamin-restricted laboratory diet alone, but when vitamins were added she never showed a loss of weight, and in many cases an actual gain. This fact would seem to suggest that on a vitamin-restricted diet the mother has to sacrifice her own tissues in order to nourish her offspring, whereas on a vitamin-rich diet the mother not only retains enough nitrogen and inorganic salts to satisfy the needs of the foetus, but actually stores it in excess, presumably as a preparation for the process of lactation. The weight at birth of the litter on the whole was not smaller on the vitamin-restricted diet, but was actually larger. The growth of the embryos *in utero* was, therefore, quite unaffected by the vitamin-supply.

After birth, however, when the animals became dependent upon the functional activity of their own alimentary canal, a difference between the two diets became apparent. Not only was there a more rapid increase in weight and size on the diet, which was supplemented with cod-liver oil and yeast, but general developmental characteristics, such as the growth of fur, were more fully advanced. These differences became even more marked after weaning, when the different diets were maintained.

These remote effects of defective nutrition, and their cumulative action through successive generations, so that the physical condition of the stock degenerates progressively, has been shown by the observations of other workers, particularly Osborne and Mendel (1912, 1913, 1923) and McCollum and co-workers (1915, 1916).

(c) **EFFECT OF DEFICIENCY IN THE YOUNG ANIMAL.**—In the young animal the effect upon growth of a vitamin deficiency can be described in three stages whose duration is variable.

According to L. Randoïn and H. Simonnet (1927) these stages are:

(1) A period of incubation, during which growth depends upon the reserve vitamins stored in the tissues, with special relation to the feeding of the mother. The duration of this period is approximately 15 to 80 days in the case of deficiency of vitamin A, 30 days in vitamin B, and 10 days in vitamin C.

(2) A period during which growth remains more or less stationary. This period corresponds to a sort of equilibrium of resistance between the needs of the organism for the deficient

substance and its liberation from the tissue reserve. The duration is shorter for vitamin C than for A or B, and depends chiefly upon the degree of the animal's development at the institution of the deficiency diet; the older the animal, the longer the duration.

(3) A period of rapid decline, leading to death, the duration depending on the vitamin reserve, and, therefore, upon the weight and age of the animal.

(d) **THE RELATION OF VITAMINS TO THE PROTEIN INTAKE.**—It has been found that the quantity or quality of protein influences the growth even when the amount of vitamins in the diet remains unaltered.

(1) **Quantity of Protein.**—A low percentage of protein in the diet has been found to produce definite ill-effects even when all other requirements were fulfilled. Examples of this inadequacy have been quoted by observers such as Reader and Drummond (1926), Stammers (1925, 1926), Suzuki (1928), Richet (1928), and many others.

The low percentage of protein in sorghum meal or "Kaffir corn" was considered by A. D. Stammers (1925) to account for the lack of growth in young rats on a diet consisting entirely of sorghum meal made into a paste with water. The average gain in weight during this period was 87 grms., as against a normal increase of 138 grms. for young rats fed on a well-balanced mixed diet. The sorghum contains 10 per cent. protein and 2.8 per cent. fat, whereas the ordinary basal diet used in rat-feeding experiments contains 22 per cent. protein and 15 per cent. fat. A second series of rats were fed with sorghum meal to which were added appropriate amounts of purified caseinogen and olive oil to bring the proportions of protein and fat approximately to the proportion in which they are present in the usual experimental diet. The gain in weight after 90 days of this diet was 122 grms. The animals remained in good health and showed no signs of deficiency disease, so that the conclusion is reached that the sorghum meal contains adequate quantities of vitamins A and B for good growth to be manifested.

Mealie (or maize) meal has been found to contain a trace of vitamin A and abundance of vitamin B. It is deficient in protein, fat, and salts, and rats fed entirely on maize meal exhibited very poor growth.

Reader and Drummond (1926), investigating the quantitative relationship between the amounts of protein and of vitamin B required to produce growth in young rats, found that good growth was obtained when the ratio of protein to yeast in the diet was 4 or 5 to 1, whether the daily consumption of protein per rat was 1 gm. or 6 grms., but poor growth resulted when the ratio of protein to yeast was 18 to 1. In a second series of experiments 70 per cent. of protein was given to all the rats, and quantities of yeast varying from 4 to 20 per cent. were added. A normal rate of growth was obtained only when at least 60 per cent. of yeast was present in the diet, and the presence or absence of butter fat made no obvious difference to the rate of growth (all rats received cod-liver oil to supply fat-soluble vitamin). In neither series of experiments was any evidence obtained that there was any quantitative relationship between the amount of yeast and the amount of carbohydrate or the total energy value of the food consumed. The high protein diets caused a hypertrophy of the kidneys only in those rats which received a diet relatively deficient in vitamin B.

Later experiments by Hassan and Drummond (1927) and Hartwell (1928) point to vitamin B₂ being more intimately concerned in the protein vitamin ratio than vitamin B₁, and the question will be further considered under vitamin B₂.

It has been shown by Frank and others that excess of protein with a vitamin-deficient diet causes an increase in the symptoms of avitaminosis, but according to Friedberger (1926), hunger and avitaminosis are not the cause of the toxic symptoms; the effects are probably only a special form of "protein toxæmia." This protein toxæmia has also been described by Tscherkes (1927), who states that the presence of fat or carbohydrate lessens the toxic symptoms in proportion to the amount present.

Suzuki (1928), investigating some artificial foods for infants, decided that it was their lack of protein which was the cause of their poor growth-promoting properties.

The addition of a vitamin B preparation caused no improvement, but a marked improvement occurred when casein, in the proportion of from 7 to 14 per cent. protein and 71 per cent. carbohydrate was the adequate ratio for normal nutrition irrespective of its vitamin content.

A diet of adequate caloric value, salt and vitamin content, found by C. Richet (1928) to produce loss of weight in a number of individuals consisting of adult brain workers, convalescent patients, pregnant women at term, and normal children (convalescent from scarlet fever). The allowance of protein was not more than 0.25 gram per kg. weight in the adult and not more than 0.54 gram in the children under 11 years. The subjects were observed over a period of 8 to 10 days. Seventeen persons lost weight (averaging 27 grms. per day), 6 gained, and 2 remained stationary.

The results were considered to show that with a deficiency of protein, even in the presence of a sufficient quantity of vitamins, not only is there a loss of body protein, but a failure adequately to utilise fats and carbohydrates.

(2) **Quality of Protein.**—The importance of the quality of the protein fraction in the diet is emphasised by several workers. Cereal proteins contain only small amounts of lysin, and success with high cereal rations, quite apart from their vitamin content, is primarily a problem of providing suitable supplements.

D. B. Jones (1925) found that the proteins of the common white navy bean will not induce growth in young animals when given as the sole source of protein in an otherwise adequate diet. The addition of 0.2 to 0.3 per cent. of cystine enables the animals to grow slowly. If the proteins are heated before they are given, and cystine is added at the same time, the young animals grow at a normal rate. These particular proteins contain more than twice as much cystine as does casein, which produces quite satisfactory growth as the sole source of protein in a diet. It would appear that for some reason as yet unknown the cystine of this bean protein is not "available," particularly when the protein is eaten uncooked.

Three proteins have been isolated from wheat bran; they contain much larger amounts of the indispensable amino-acids, especially lysine, tryptophane, and cystine, than do the proteins of the wheat endosperm, which are found in white flour. Young rats fed on a diet containing abundant supplies of vitamins A and B and bran as the sole source of protein grew much better than those fed on wheat endosperm with the same amounts of vitamins.

Similarly V. G. Heller (1927), investigating the nutritive properties of the mung bean, found that, though its protein content was high, with a fair distribution of the nitrogen it was not adequate as a sole source of protein.

Rats on a diet in which the beans, at a 60 per cent. level, formed the sole source of protein, grew at a rate somewhat below normal and reproduction was limited. With the beans forming 90 per cent., the ration was improved, and with the addition of 1 per cent. cystine, third generation reproduction was obtained. The addition of cod-liver oil to the diet made no difference, so that vitamin A is present in greater amount than in most seeds. The beans, at a 60 per cent. level, form a plentiful source of vitamin B, and the addition of NaCl and CaCO₃ completes the mineral requirements.

If cereals are given in high percentage as the source of calories the basal diet must contain supplementing protein. Thus, with a basal diet containing meat residue, dried cooked liver, salt mixture, with a daily addition of fresh lettuce, Cowgill, Jones, Frisch, and Jackson (1927) found that excellent growth (above normal), reproduction, and general health resulted when whole-grain cereals or a breakfast milled-wheat food-product formed as much as 84 per cent. of the calories of the diet. A milled-corn product, such as hominy, was only slightly inferior to these products, whilst dextrinised starch produced excellent growth at the

level of 76 per cent., but less vigorous growth at an 84 per cent. level. No rickets was observed in any animal. (The diets contained 2 per cent. of cod-liver oil.) Diets containing as much as 93 per cent. whole-grain calories allowed of growth approximating the "normal."

The growth-promoting properties of these diets are attributed to the nature of the basal diet and confirm the observations of Osborne and Mendel concerning the value of certain combinations of liver and lettuce in this respect.

Young rats were successfully reared on diets containing 65 per cent. of whole grain cereals such as oats, wheat, or maize when the remainder of the food consisted of eggs, molasses, and fresh lettuce. When the amount of cereal was increased to 93 per cent. normal rate of growth was not obtained, the limiting factor being apparently the small amount of protein in the diet.

The inferiority of a diet containing cereal protein as its chief protein constituent in a mixed diet has been shown by the experiments of McCarrison (1927) on the comparative nutritive value of wheat, paddy, and certain other food grains, and by those of Mottram on the nutritive value of bread.

McCarrison found that the addition of wheat to a basal diet deficient in vitamins A and B produced much better growth than the addition of paddy, while Cambu (*Pennisetum typhordeum*) and Cholan (*Andropogon sorghum*) produced an intermediate degree of growth. Cod-liver oil and marmite together supplemented the basal diet just about as well as whole wheat, but still better growth occurred with a mixed diet containing wheat, milk, fresh green vegetables, and meat than with any of the "artificial" diets employed.

Mottram and Cramer's (1928) experiments on the proteins of bread confirmed the desirability of a mixed diet, and the high biological value of animal proteins supplementing the cereal. They found that the inadequacy of bread was not due mainly to its lack of vitamins A and D, to its poverty in vitamin B, nor to its lack of mineral matter. Young rats put on a diet of bread, mineral matter, butter, and marmite grew very slowly—approximately 1.42 grms. per day. Such a diet is adequate as regards vitamins A, B, and D, and as regards salts. Others on a mixed diet grew at the rate of 4.7 grms. per day, or about three and a quarter times as fast.

The protein deficiency was tested—

(1) By adding gluten, the mixed proteins of white flour ;

(2) By adding an acknowledged first-class protein such as casein, to a bread, salt, and vitamin diet, and observing the growth of the young.

Since the addition of gluten improved the rate of growth, bread is shown to be deficient in the quantity of its protein (8 to 10 per cent.). Since on adding casein a further improvement occurred the protein of bread is deficient also in quality. The addition of proteins with relatively large amounts of lysin present in their molecules is necessary to supplement those of bread. Consequently, the addition of gelatin, which, according to Mottram and Robinson, is by itself a useless protein, results in a mixture which has high food value. White bread supplemented with gelatin enabled young rats to grow better than white bread *plus* gluten. The value of gelatin in this connection is also borne out by the investigations of Hess and Chamberlain into the diets of artificially fed infants. Twenty-eight infants on artificial diet were given gelatin or egg yolk-orange juice in addition to milk. A study covering more than 150 weeks on each mixture showed an average gain of 6.44 and 6.46 ounces per week respectively.

In Mottram's experiments the inadequacy of bread was shown not only in the growth rate of young animals but in the deficiency of their coats. They became bald for a time, though they ultimately grew hair again. Their final coats on a bread diet were never so thick as when the animals received a control mixed diet. The fault lay with the low quantity of

protein in the bread, for addition of extra gluten prevented the occurrence of baldness. So, too, did casein and gelatin, though addition of vitamin B did not. An allied result was the paling of the pigmented areas of the animals' coats. Rapid growth with poverty of diet led to greying of the hair, which darkened again very soon if satisfactory protein, such as casein, was supplied.

A further interesting and significant fact is that the inadequacy of bread shows more clearly with males than with females. Normally the male rat is larger than the female, but upon an inadequate diet, such as bread, his growth rate is smaller. This is partly due to the nature of the protein, for if an animal protein is added to the diet the male regains his normal superiority, while if only gluten be added he does better than on bread alone, but only equals the female in weight. (It is interesting to note that gelatin supplements the proteins in bread in this respect too—the combination of two proteins, both poor, results in a pabulum which, if not quite first rate, is better than bread *plus* gluten.)

The extra growth of the male is dependent on another factor as well—vitamin B. The addition of a preparation from yeast containing no protein (though amino-acids are present) enables the male to resume his normal comparative stature. Perhaps it is the co-operation of these two factors (better protein and more vitamin B) which accounts for the slightly improved growth when brown bread is used instead of white in experiments on malnutrition.

Investigation by G. A. Hartwell (1921, 1922) of the growth rates of suckling rats when their mothers were on different simple diets showed that bread and meat was nearly equal in value to bread and milk, while bread alone, bread and butter, bread and dextrin, were far behind. Meat alone gave a very poor growth which may have been due (1) to the impossibility of eating sufficient bulk of food, or (2) to the lack of balance between the excess of protein and the vitamin B in the diet. When a mixture of one part by weight, or even less, of protein to three parts of bread was given, the young grew well for about 10 days and then began to develop tetanic spasms and convulsions, usually ending in death.

These results can be entirely avoided by giving large quantities of milk in the diet, and an extended investigation proved fairly conclusively that it was the vitamin B content of the milk which obviated the trouble. Any preparation which contains vitamin B in considerable amount, such as marmite, juices of potato, tomato, or carrot, wheat germ extract, or soya bean extract, will prevent untoward happenings. It has been shown that, to bring up offspring successfully, when there is much protein in the diet the nursing mother needs three to four times as much vitamin B as is necessary for growth and reproduction.

Hartwell (1928), Reader and Drummond (1928), agree that the explanation lies in the fact that vitamin B is essential as a catalyst for excessive protein metabolism (see vitamin B₂).

The proteins of shell-fish have been found by D. B. Jones (1926) to have varying nutritional values apart from their vitamin content.

The proteins of oysters, which are a fairly good source of both vitamin A and vitamin B given to young rats in the proportion of 9 per cent. of the total ration, produced fair growth, but not as rapid as the protein of clams and shrimps fed in the same proportion.

Finally, there is the complicated problem of the relationship between growth and protein and vitamins introduced by the work of Palmer and Kennedy (1927). In studying the effects upon growth of varying sources of protein, they found that improvement took place when impurified proteins were substituted for the purified casein hitherto employed. It would have been an obvious conclusion that the purification of the proteins had removed the vitamin content, and therefore their growth-promoting power, had it not been for the fact that other experiments performed at the same time seemed to show that extra growth was not produced by the addition of vitamins to the purified casein. Thus, additions of vitamins A, B, C, D, and E had been made, singly and in combination, without success. They were therefore led

to the tentative conclusion that the quality of the protein in the diet has an effect quite apart from that of vitamins, and that impurified proteins from animal products may carry growth-promoting factors other than the recognised vitamins of the water and fat-soluble groups.

From later experiments the suggestion has been made that the factor removed by purification may be one which links the vitamin and the fat into an intracellular lipin.

Rosenbloom has suggested that fat is normally present in tissues in a "masked" form as a calcium-protein-fat compound. If the fat remains free it is possible that vitamin A, not linked with fat, shows an antagonistic attitude towards vitamin B.

IX. VITAMINS AND BACTERIAL GROWTH.

The question of the relations between vitamins and the growth of bacteria has led to some controversy, and of the results obtained by several investigators, none go much further than the suggestion that the presence of substances analogous to vitamins in the culture media tends to increase the growth of certain micro-organisms.

D. J. Davis (1917, 1918), investigating the growth of hæmophilic bacteria, found that for maximum development two factors must be present in the medium: the first, pure hæmoglobin, and the second, some substance which resides in foreign bacteria and in fresh animal and plant tissue. He pointed out the similarity between vitamin action and that of this unknown substance, but was unable to explain its mechanism, except by the suggestion that the unknown substance might act by making the iron in the hæmoglobin available for bacterial assimilation. Further studies on these lines have been undertaken by Kollath (1926), who calls the two factors necessary for growth "X" and "V." He states that both factors are found in red blood cells, and that blood serum contains another vitamin-like substance which destroys the V factor. In plant extracts both factors can be eventually obtained, but the V factor, heat labile, is found much earlier than the X factor, heat resistant and active in small amounts. On the basis of the above hypothesis, Kollath has suggested an explanation of the fact that *B. influenza* forms "giant" growths when cultivated near colonies of other bacteria in media containing small amounts of blood. He states that the associated bacteria in such conditions produce the V factor which stimulates *B. influenza* to excessive growth.

Testing the blood serum of animals with scurvy he found an increase of the vitamin-like substance which destroys the V factor, its amount being in proportion to the severity of the disease, but the red cells were unaffected, and no such substance was found in pigeons suffering from beri-beri. He concludes that the vitamin which stimulates bacterial growth is not identical with vitamin B and C, but that it has some significance in connection with problems of the water-soluble vitamins.

This conclusion is reminiscent of that reached by Husoya and Kuroya (1923), who divided bacteria into two groups: (1) *Streptococcus hæmolyticus* and *pneumococcus*, requiring some unknown water-soluble substance for their growth. (2) Most other bacteria tested, including meningococcus, for which the substance was not absolutely necessary.

Attempting to discover substances that might stimulate the growth of bacteria, Davis employed *B. coli*, *B. typhosus*, *B. diphtheriæ*, *B. pyocyaneus*, *B. prodigiosus*, Blastomycetes, Sporotrichum, Streptothrix, *Streptococcus hæmolyticus*, and *Staphylococcus aureus*. None of these appeared to be stimulated by the presence of hæmoglobin added to ordinary mediums. Distilled water containing 1 per cent. pure hæmoglobin did not support significant growth. Mediums containing 5 per cent. by weight of rice flour, white wheat flour, and whole wheat flour (also NaCl and agar) supported good growth in all cases, but less than on plain meat extract agar. When these cereals were added to meat extract agar the cultures grew but showed no increase in profuseness of growth. He concluded, therefore, that the vitamin substances in grains were not of significance when influencing the growth of the culture studied.

On the other hand, when a flour was prepared out of dried, sprouted grains (rice and wheat) and added to mediums, all the bacteria grew more rapidly and profusely, suggesting that constituents made soluble by the sprouting process were responsible for the stimulating effect, a supposition strengthened by the fact that filtering the medium removed some of the growth-promoting factors.

In experiments carried out by L. J. Kligler (1919) the pathogenic organisms tested were gonococcus, meningococcus, Type 1 pneumococcus, *Streptococcus hemolyticus*, *B. diphtheria*, *B. pertussis*, and *B. influenza*, and the media used were extracts of fresh animal tissues and human secretions, added to nutrient broth or phosphate peptone agar.

Typhoid and Shiga Flexner dysentery were included in the early experiments, but were later excluded because they grew equally well on the control and the extract media. It was found that extracts of beef heart, rabbit and cat tissues, as well as human nasal secretions, contained substances favouring the growth of all the organisms tested. The mucosa of different organs, together with spleen, liver, and kidney tissue, was found to be especially rich in these substances, while the muscle was relatively poor; the favourable substance, whatever its nature, apparently being present in higher concentration in the mucosa than in muscle. Saline and alcoholic extracts of human nasal washings also enhanced growth, the saline extracts being the more efficient. In order to ascertain the relative significance of the fat-soluble A and water-soluble B in the cultivation of these micro-organisms, comparative tests were made with saline and ether extracts of blood-clot rich in vitamins.

It was found that the saline extract contained substances favourable to the growth of bacteria, while the ether extract apparently lacked them.

It was shown that removal of the protein fraction of the extracts by precipitation with alcohol did not inhibit their favourable effect on bacterial growth, and that whereas amino acids or carbohydrates, the essential nutritive substances for bacteria, are not affected by heat, the organ extracts were decidedly injured by heat—a fact which supports the view that they belong to the class of growth accessory substances.

Activating substances were separated from beef-heart infusions and autolysed yeast solutions by means of fuller's earth and norite, according to the method of Funk and Dubin (1920), and the activated adsorbents, having been extracted with baryta and glacial acetic acid respectively, were tested on the growth of yeast cells and streptococci. Freedman and Funk (1922) concluded from the results of these experiments that the substances thus extracted from beef heart and autolysed yeast solutions apparently belonged to the class of water-soluble vitamins, Type B, but were not identical with it, though showing similar properties. The action of protein hydrolysates (animal and vegetable proteins, freed from vitamins and subjected to acid hydrolysis) was tested on the growth of streptococci and yeast cells, the growth being measured by the acidity of the medium.

The investigators suggest that the results on streptococci indicate the presence in the protein hydrolysate of a vitamin-like substance, probably similar to vitamin B, and present in an impurity which could not be removed by the known methods of protein purification.

Results which they suggest were "compatible with the vitamin hypothesis" were obtained by McLeod and Wyon (1921), using as media watery and alcoholic extracts of organic materials such as kidney, liver, egg-yolk, yeast extract (marmite), muscle, milk, human serum, etc. Their potency in giving growth for *Staphylococcus aureus* was compared with that of substances lacking B vitamin such as casein, glucose, glycerine, etc., the figures varying from a potency of 2000 for guinea-pig kidney, and 500 for yeast extract, to 3.3 for casein, and 1 for glucose. Comparison of the amino-acid content with the potency showed that the results were not entirely due to amino-acids.

On using organic substances (blood serum, whole egg, marmite) for culturing pneumococcus and meningococcus the following conclusions were drawn :

1. The growth-promoting power does not bear any definite ratio to the known vitamin content, since marmite, known to be the richest in vitamins, had little or no effect, while charcoal, certainly devoid of vitamins, had quite an appreciable effect.

2. The growth-promoting power for pneumococci is to some extent destroyed by heating serum, etc., while that for meningococcus is little altered or increased.

Active tryptic or peptic digestion of these substances was found to destroy completely their power of promoting the growth of pneumococci or meningococci. Since known vitamins must pass to the body by way of the stomach and duodenum, where they are subjected to the digestive juices, it seems that they must differ in nature from the growth-promoting factor for these bacteria.

No satisfactory evidence could be obtained that the growth-promoting factor for these bacteria could be extracted with alcohol.

With regard to the growth of tubercle bacilli, according to Nao Uyei (1927), it appears that the presence of vitamins A and D exerts an indifferent effect, but that substances rich in vitamin B (Harris's yeast vitamin, orange, tomato, and cabbage juices) exert a pronounced stimulation.

On the other hand, it was pointed out by Ayers and Mudge (1922), who also found cabbage extract to promote the growth of a pathogenic streptococcus, that the effect might be explicable on the grounds of its sugar content, since equivalent concentrations of glucose are equally effective. Fats and oils of animal, mineral, and vegetable sources stimulated the growth of the streptococcus, as also did autolysed yeast extract. Most yeast organisms appear to be able to grow in media which contain little or no vitamin, and although enrichment by organic vitamin-containing substances does produce more luxuriant growth, it is suggested by Lumière (1921) that the effect is due to the substances accompanying the vitamins rather than to the vitamins themselves.

A yeast isolated from fermenting canned pears by Freda M. Bachmann (1919), however, had not the power of causing fermentation of a medium which contained little or no vitamin, nor was it capable of synthesising its own vitamins. A substance which promotes the growth of *Streptothrix corallinus* was suspected of being identical with vitamin B, but tests by Peters, Kinnersley, Orr Ewing, and Reader (1928) have shown that this substance is not identical with either B₁ or B₂.

Experiments by Werkman (1927) have shown that vitamin B does not stimulate multiplication of *Azotobacter chroococcum* and *Rhizobium leguminosarum*. Even when many more organisms were present in a culture containing a concentration of a vitamin B preparation as high as 1 : 10,000 than were present in the control flask, rates of multiplication were the same in the two flasks. Werkman explains the occurrence of a greater number of organisms in the vitaminised flask by the suggestion that the initial stimulation is due to small amounts of readily available food material other than vitamin B. When this foodstuff is exhausted, multiplication goes on at the same rate as in the control flask. He considers, therefore, that the term vitamin should not be extended to include substances necessary for the growth or the reproduction of micro-organisms.

The revivifying effect of a vitamin extract containing water-soluble B on old cultures of various species of organisms was demonstrated by Terrigo and Barnett. Nearly two-thirds of the 30 species tried showed marked increase of growth due to the vitamin extract additions, the increase varying not only with the organisms but with the length of the vitamin treatment.

Sclerotinia cinerea, the known rot fungus of peaches and plums, has, according to J. J. Willaman, shown growth on media to which small amounts of plant decoctions and vitamin

preparations from a large number of sources had been added. All these preparations were active in promoting growth, and a few would promote reproduction as well.

Fungi, such as *Oidium lactis*, according to Linossier (1919), grow much more abundantly in a vitamin-rich medium, though *Oidium lactis* is one of the monocellular organisms which can apparently do without vitamins. The organism was therefore subjected to conditions which would lower its vitality (old cultures, heating to a certain degree); in sufficiently weakened conditions vitamins were found indispensable to growth, in a lesser degree growth was inhibited by the absence of vitamins. The vitaminising agents used were macerated cabbage leaves and orange juice.

In Menkes' (1927) experiments on the action of vitamins on fungi, the vitaminising agent used was an alcoholic extract of tomatoes. He noted differences in colour, odour, and acidity between the vitaminised flasks and the controls, and an increase in the absorption of sugar in the presence of vitamins, especially at the beginning of growth. There seemed to be a relation between the quantity of sugar absorbed and the weight of the fungus. From these observations Menkes deduces that sugar alone is assimilated more strongly in the presence of vitamins, and that there is excitation of growth and assimilation in general.

X. PRODUCTION OF VITAMINS BY BACTERIA.

Experiments have been undertaken by several workers in the attempt to discover whether bacteria during their growth produced any vitamins, but the concensus of opinion seems to point to no such production.

Wollman and Vagliano (1922) failed to produce vitamins A, B, or C by the cultivation of either *B. bulgaricus*, *Amylomucor*, or *Amylobacter*, and Slanetz (1923) was equally unsuccessful with various pathogenic and soil-growing organisms, such as *B. lactis acidii*, *B. mycoides*, *B. subtilis*, *B. prodigiosus*, etc.

R. C. Robertson (1924), however, has shown that synthetic media which refused to support the growth of yeast cells, became capable of stimulating their growth after *B. coli* had been cultivated on them. From this result he inferred that *B. coli* apparently generated some substance or substances during growth which permitted the growth of yeast cells. Husoya and Kuroya (1923) also found that *B. coli* could synthesise a water-soluble substance necessary for the growth of certain bacteria. In Robertson's later experiments other organisms besides *B. coli* were used, including *B. proteus*, *B. pyocyaneus*, *B. subtilis*, and *Sarcina lutea*, and rigorous filtration, titration, and autoclaving of successive generations were carried out, so that any growth-stimulating substances carried over by the first inoculation would, before the end of the experiments, be diluted to such an extent as to be practically negligible.

Robertson concludes that all these non-pathogenic organisms do produce substances which are of the nature of growth-stimulating substances, but is not prepared to say that they are capable of continuous growth on or of producing growth-stimulating substances in all vitamin-free media.

P. Goy (1925) states definitely that a substance which favours the growth of yeasts and bacteria is present in the cultures of *Amylomucor B.*, but asserts that this substance is not identical with vitamin B, its resistance to high temperatures (1½ hr. at 130° C.) distinguishing it from vitamins of both animal and vegetable origin.

S. R. Damon (1922, 1923) has also postulated a growth-stimulating substance analogous to vitamin B in cultures from at least three members of the group of acid-fast bacteria—the Timothy bacillus, *B. smegmatis* and *B. mælleri*, and Scheunert and Schiebllich (1927) have concluded from experiments with *Bacillus vulgatus* that this organism has the power to form vitamin B out of a nutrient solution known to be free from this vitamin.

An extensive investigation into the synthesis of vitamin B by various genera of micro-

organisms has recently been undertaken by Gertrude Sunderlin and C. H. Werkman (1928), the organisms used purposely including some such as *Bacillus adhærens*, *Torula rosea*, and *Oospora lactis*, which had previously been reported as incapable of synthesising vitamin B.

Their conclusions are reported very definitely as follows :

“ The synthesis of vitamin B by such biologically separated genera of micro-organisms as *Torula*, *Oospora*, *Actinomyces*, and four genera of the order *Eubacteriales* reveals a general occurrence of vitamin B synthesis among widely separated groups of the lower plant forms. Vitamin B, whatever its structure chemically may be, is a constituent prevalent in micro-organisms.

“ Specifically the following organisms were found to produce the growth-promoting vitamin for white rats : *Torula rosea*, *Oospora lactis*, *Bacillus adhærens*, *Bacterium coli*, *Bacillus subtilis*, *Bacillus mycoides*, *Azotobacter chroococcum*, *Rhizobium leguminosarum*, and *Actinomyces* (species unknown).

“ The results showed no marked differences in the ability of 3 strains of *Bacterium coli* to synthesise vitamin B.

“ Drying at 37° C. or at 100° C. for 48 hours does not materially diminish the vitamin potency of the bacterial mass.”

Several reasons are suggested to account for the discrepancies present in the literature dealing with the synthesis of vitamin B by micro-organisms. Our conceptions of a vitamin are not in accord and thus differently defined the term vitamin assumes an ambiguous and obscured meaning until it is in direct conflict with the original conception intended.

After limiting by definition the meaning of the term vitamin to the legitimate conception expressed by Drummond we find a multiplicity of methods employed to determine the synthesis of a vitamin by micro-organisms. Important among these are—(1) quantity of organism fed ; (2) period of feeding and time allowed for the rats to become accustomed to the bacterial diet ; (3) manner of feeding organisms to the rats ; (4) species of animal used as an indicator.

Vitamin C is apparently not synthesised by bacteria to any considerable degree. Kollath and co-workers (1926) investigated colon bacilli, Friedländer pneumobacilli, and mixtures of intestinal bacteria were studied. Nutrient agar and a nutrient medium containing a plant extract with V substance were also fed. The last named alone showed antiscorbutic properties.

VITAMINS PRODUCED BY BACTERIA IN SILAGE.—Silages prepared by both the “ cold ” and the “ hot ” processes from alfalfa, red clover, saradella, vetch, grass, maize, turnip leaves, sugar beet leaves and tops, beans, peas, barley, and oats, have been tested by Scheunert (1927) for their vitamin content. A few specimens were 2 years old ; others had been kept 1 year ; presumably the rest had been stored only a few months. In studies of vitamins A and B, the rat growth method was employed. Guinea-pigs were used in studies of vitamin C, and the prophylactic method followed. As to the vitamin A content of the silages, usually 15 grms. (daily) sufficed for restoration of health and resumption of growth. The activity of the various preparations did not vary much with the method of preparation. The quantity of vitamin B seemed quite variable, and more silage was required to supply sufficient vitamin B than was necessary to supply the optimum amount of A. In no case was growth satisfactory on less than 2 grms. (daily). In one trial 8 grms. was insufficient. In general, legumes contained more vitamin B than did the other materials. Fewer trials were conducted with vitamin C, partly because the guinea-pigs often refused to consume sufficient quantities. Various specimens of silage contained vitamin C, though no doubt in reduced amount. In some cases 4 grms. of silage daily per 100 grms. body weight supplied a sufficient amount, in others 10 grms. were necessary.

XI. DISTURBANCES OF HEALTH DUE TO NON-SPECIFIC VITAMIN DEFICIENCY.

With the discovery that specific diseases such as beri-beri and scurvy were associated with vitamins, each having their characteristic properties and reactions, a tendency arose to regard vitamins as almost entirely specific in action. Vitamin A, for instance, has been called the anti-ophthalmic vitamin, and vitamin D the antirachitic, while vitamin E has been definitely attached to the function of reproduction in both the male and the female.

As the scope of investigation into the biological significance of vitamins has extended, however, it has become obvious that, though specific in many respects, the effects of deficiency of the various vitamins show overlapping, and are not confined exclusively to the production of a definite and characteristic symptomatology.

(a) **GENERAL DISTURBANCES OF HEALTH.**—The production of ailments other than disturbances of growth, on the one hand, and the recognised deficiency diseases due to lack of the specific vitamins, on the other, was recognised fairly early in the course of investigations into avitaminosis.

McCarrison, in 1921, stated that faulty food deficient in vitamins was a common cause of such human ailments, as diarrhoea, constipation, chronic gastro-intestinal dyspepsia, gastric and duodenal ulcer, mucous disease, colitis, and chronic gastro-intestinal stasis—maladies which are “among the commonest complaints of civilised man,” since they account for about one-quarter of his total illnesses. He has also pointed out that the question of vitamin deficiency, especially in countries whose inhabitants exist on a mixed diet, is one of partial rather than complete deficiency.

“There is no such thing in nature as a diet wholly devoid of one vitamin while complete in every other way. Always when there is deficiency of one vitamin there are other food faults besides: deficiency of another vitamin, or of inorganic salts, or of suitable protein, or excess of carbohydrate, or ill balance of the diet in other respects. Consequently, those of man’s ailments—especially his gastro-intestinal ailments—which are due to his faulty food, are the result of the peculiar combinations of its faults, together, in many cases, with the superimposed effects of pathogenic agents. It is the faulty food deficient in vitamins which results in “depreciation of cellular function, and depreciation of cellular function is the foundation upon which disease is built.”

McCarrison’s experiments (1926) on rats on a diet designed to represent the partially deficient diet of many Western people of the poorer classes were illustrative of these principles. The mortality of the group whose diet consisted of white bread, vegetables cooked in the presence of alkali, coco-nut oil with boric acid added as a preservative, tinned meat, tinned jam, and tea, was 45 per cent. as compared with 15 per cent. in the colony on a diet of whole wheat flour, uncooked vegetables, fresh fruit, butter, and fresh milk.

The survivors in the first colony were well grown, sleek coated, strong and active; those in the second colony were ill-grown, poor coated, weakly and listless.*

(b) **GASTRO-INTESTINAL CHANGES.**—Detailed investigations into the intestinal lesions of animals fed on vitamin deficient and otherwise unbalanced diets have been made by McCarrison (1919, 1923). The most constant and important post-mortem findings were changes in the gastro-intestinal tract which impaired its functional capacity, dilatation, and thinning of the whole tract; atony and narrowing of the lumen of the bowel; intestinal stasis; and an abnormal growth of epithelium in the proximal portion of the rat’s stomach.

In other experiments on monkeys, fed on a diet whose vitamin value had been reduced by autoclaving to a sufficient degree of heat, the animals soon began to show signs of impaired

(* From “A Good Diet and a Bad One,” McCarrison, *Brit. Med. Journ.*, 23rd October 1926.)

health. They survived for periods ranging from 51 to 100 days ; during this period they lost weight, and their appetites became impaired. The most constant and one of the earliest symptoms from which they suffered was diarrhœa, either with or without mucus and blood in the stools. Dysentery without preliminary diarrhœa developed in 5 of them ; diarrhœa alone in 4 ; so that of the 11 animals fed in this way, 9, or 81 per cent., exhibited a severe intestinal flux, while the 8 control animals, living in the same room, and having the same food which had not been autoclaved, remained free from these disorders, and indeed in good health generally (after a dose or two of Epsom salts had freed them of their jaundice, due, it was thought, to sudden restriction of their active habits and to the overeating of ground-nuts). Of the 5 animals which suffered from dysentery some were found to have cystic and active forms of *Entamœba histolytica* in the stools, the active forms containing ingested blood corpuscles. Others had amœbæ also, but unless these were found to contain blood corpuscles the diagnosis of amœbic dysentery was not made. Examination of the 8 controls showed that 2 of them were " carriers " of entamœba cysts. The conclusion was accordingly reached that the animals which presented amœbic dysentery had also been " carriers " of this organism when the experiment was begun, and that the amœba had remained harmless to the well-fed animals while it attacked those the food of which was deficient in vitamins. No bacteriological examination was made to ascertain the nature of the dysentery, which was not diagnosed as amœbic, or of the diarrhœa ; but it can hardly be doubted that bacterial agents were concerned in their production. The first effect, then, of food deficient in vitamins is to render the intestinal tract, and more especially certain parts of it, susceptible to invasion by bacterial or protozoal agents. It renders pathogenic to the ill-fed host organisms that may be non-pathogenic to the well-fed host.

The gross anatomical lesions found in these animals, which were more pronounced and more frequent in those animals whose food was deficient in vitamin A as well as vitamin B, were as follows : (1) Enlargement of the mesenteric glands ; (2) dilatation of the stomach ; (3) congestion of the gastro-intestinal tract with ecchymoses scattered over its peritoneal surface, more commonly present in the colon ; (4) ballooning of various parts of the tract, especially of the colon ; (5) marked thinning of the muscular walls of the colon and partial disappearance of its longitudinal muscular bands. On opening the tract the following pathological changes were observed : (1) Congestion and ecchymosis of the stomach, especially in the region of the pylorus, were present in 6 cases ; shallow ulcers of the size of a threepenny-piece were present in 2 cases ; in another, papillary downgrowths of epithelium, suggestive of a commencing carcinoma, were found in the region of the pylorus. (2) Congestion and ecchymosis involving part or whole of the small bowel were occasionally present. (3) In the colon the changes consisted in greater or lesser degrees of colitis, sometimes involving the whole viscus, sometimes part of it : colitis, which is one of the commonest consequences of food deficient in vitamins, was the most marked and one of the most constant of the morbid anatomical changes present. On histological examination the changes found in various parts of the tract included : (1) Congestion and hæmorrhage ; (2) atrophy of the muscular coats of the bowel, especially prominent in the colon ; (3) degenerative changes in the myenteric plexus of Auerbach, sometimes limited to a few ganglia ; (4) atrophic, necrotic, and inflammatory changes in the mucous membrane of the entire tract, particularly marked in the colon ; (5) bacterial invasion of the bowel walls frequently extending deep into the coats of the viscus ; (6) the opening up of portals of entry by which bacteria might enter directly into the blood stream ; and (7) pronounced atrophy of the lymphoid elements of the intestinal mucosa.

In his later work (1927) on the effects of faulty food deficient in vitamins on the gastro-intestinal tract, McCarrison makes a distinction between the action of vitamins B₁ and B₂.

He states that antineuritic vitamin B₁ is probably associated with the normal motor activities of the stomach and intestines whilst deficiency of the antipellagra vitamin (B₂) is the chief cause of the gastro-intestinal lesions found in this disease.

The general effects may be briefly summarised as follows: (1) alimentary dystrophy; (2) impairment of digestive and absorptive processes; (3) congestive, necrotic, and inflammatory changes in the mucous membrane; (4) degenerative changes in the neuro-muscular mechanism; (5) degenerative changes in the secretory glands; (6) toxic absorption from the dystrophic bowel; (7) pronounced reduction in the number of lymphoid cells throughout the tract; (8) impairment of the protective power of the mucosa against infecting agents (bacterial and protozoal), resulting in infection of the bowel wall and the passage of bacteria into the blood stream.

Gross (1924), working also with rats, and using synthetic diets deficient in vitamin B, has demonstrated *in vivo* and *in vitro* that pronounced impairment of the motor functions of the intestinal tract, due either to atrophy or other qualitative changes in the muscular or neuro-muscular apparatus, results in consequence of want of this vitamin, but he was unable to confirm McCarrison's findings in respect of degeneration of Auerbach's plexus.

In direct contradiction to these results, on the other hand, are those of Gudjónsson (1927), who has investigated the rate of travel of the intestinal contents in rats on normal and experimentally defective diet. This investigation was suggested by an observation of Fredericia, in 1926, that the fæces from "refected" rats are abnormally voluminous and contain much undigested starch. The investigation was based on the possibility that the rate of travel in the intestine was so rapid as to leave no time for absorption to take place.

Gudjónsson used the carmine method in his experiments; the carmine was given with saccharin and gelatin, and flavoured with a drop of anise. The mixture was well taken by the animals, and they were liberally fed with it for a considerable time, in order to establish the normal condition of the intestinal functions. Data of measurement were taken by means of a device called a "stercograph" consisting essentially of a revolving disc of cardboard divided by radiating lines into 21 numbered segments, the disc being arranged to make one complete revolution in 24 hours. Above the disc is suspended a wire cage containing the rats, and below the cage are two inclined zinc plates with an opening at their lower margin through which the fæces fall into the revolving disc. The first evidence of coloured fæces is noted and, in difficult cases, a little of the latter may be dissolved in water, and potash added, drop by drop, until a deep violet-red colour is produced. Examination of the disc shows the number of hours which elapse from the first taking of the carmine food, the number of defæcations in 24 hours, and the number of fæcal lumps. The fæces were weighed with the usual precautions and the water-content determined. The results of the investigation showed that rats suffering from beri-beri have a distinctly more rapid passage of the intestinal contents but a lessened water-content. Vitamin A-deficient rats showed slightly increased rapidity but no really significant divergence from the normal, the water-content being rather higher than normal. "Refected" rats showed a more rapid passage but less water-content than normal, and no intestinal stasis was to be found in rats suffering from the effects of A- and B-vitamin-free diets. On the contrary, the motor functions of the intestine seem to have been increased and in vitamin B-deficient rats increased to a very marked degree.

In 1924 Pappenheimer and Larimore recorded the occurrence of gastric lesions in 55 per cent. of rats fed on synthetic diets in which the only known deficiency was want of vitamin A. They found that the addition of cod-liver oil to these diets afforded complete protection against these gastric lesions.

Although they kept in mind the possibility that other factors such as the ingestion of hair, or of some infective agent present in the hair, might have some share in the production

of the gastric lesions, Pappenheimer and Larimore came to the conclusion that these lesions were definitely related to vitamin deficiency.

Gastric and duodenal lesions due to deficiency of vitamins A, C, and D have also been reported by Magee, Anderson, and McCallum (1928, 1929).

Guinea-pigs (three-fourths grown) adrenalectomised unilaterally and also unoperated were fed on diets deficient in vitamins A, C, and D, and in Ca, Na, and Cl. They were killed as soon as symptoms of malnutrition became well established (after about 4 weeks). The majority showed variously severe circulatory or degenerative lesions (ulcers and erosions) in the pyloric region of the stomach. The longer the animals lived, the more marked were the lesions. The adrenals also showed congestive changes or excessive vacuolation of the cortex. A majority of unilaterally adrenalectomised animals fed on complete diets had lesions like the above in the remaining adrenal, and some showed hæmorrhages in the gastric mucosa, but growth was not affected.

The suggestion is made that disorganisation of intestinal rhythm, alimentary stasis, abnormal putrefaction, and adrenal exhaustion are stages in the development of the gastric and duodenal lesions.

The high incidence of abdominal infections in America is attributed by Harris (1928) to the fact that a large proportion of the American people consume an excess of sugar and other carbohydrates which are deficient in vitamins. He points out that in McCarrison's experiments with monkeys a diet poor in vitamins and rich in carbohydrates rendered the animals very prone to gastro-intestinal infections, including gastric ulcer. He believes that a large percentage of cases of gastric and duodenal ulcers could be cured by a diet rich in every vitamin.

Cramer (1920), using rats fed on synthetic diets deficient either in vitamin A or in vitamin B, found that digestion of fats was less complete or less rapid in the absence of vitamins; that the effect on fat-absorption was an immediate one; and that vitamins A and B, especially the latter, produced a definite stimulating action on the processes of intestinal digestion and absorption. He attributed to want of vitamin A alone the loss of the protective mucus covering the mucous membrane; this is an important factor in facilitating the bacterial invasion of the mucosa which he also observed in his animals; and he attached to this deficiency an important share in causing the impairment of the absorptive function of the mucous membrane.

(c) **VITAMINS AND INTESTINAL WORMS.**—The relation between vitamin deficiency and infestation of the alimentary tract by worms has been investigated by Sasaki (1928). He has reached the following conclusions: (1) Although infestation by worms is caused by ingesting them in some stage of their life cycle, the extent of infestation does not necessarily depend on the number ingested; (2) a lack of vitamins A and D makes infestation easy; (3) a lack of vitamin B contributes to early infestation; (4) a lack of vitamin C lowers the rate of infestation, while an excess of vitamin C increases it; and (5) the fact that the rate of infestation is in proportion to the lack or excess of vitamins depends on the amount of bile that is secreted.

(d) **CHANGES IN LYMPHOID AND BLOOD-FORMING TISSUE.**—Cramer (1921) found that lymphoid tissue was profoundly affected by the absence of vitamins from the diet. In mice and rats kept on a diet completely free from vitamins there is a great atrophy of the lymphoid tissue, which is obvious even to the naked eye. The spleen is shrunk to a narrow ribbon; the thymus, which even in adult rats and mice remains relatively a large organ filling the upper part of the thorax and covering the base of the heart, becomes so small as to be hardly visible. The Peyer's patches, which are prominent in normal animals, are difficult to identify with the naked eye. Microscopically these organs, especially the spleen, are found to contain very few leucocytes. The ordinary lymph glands may not be macroscopically smaller than normal, but under the microscope are found to be almost bare of lymphocytes. They consist almost entirely of endothelial cells and large empty lymph spaces. The blood picture, as

shown by a differential count, shows a reduction in the number of lymphocytes and an increase in the number of polymorphonuclear leucocytes.

Extreme Reduction of Number of Lymphocytes.—As an example of the degree to which the lymphocytes may be affected, the following extreme figures obtained in a mouse and a rat in the final stages of avitaminosis may be given. These animals did not suffer from any intercurrent infection :

	Lympho- cytes.	Large Mono- nuclears.	Poly- morphs.	Mast Cells.	Eosino- philes.
	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.
Avitaminous rat No. 130 ...	25	15	57	2	1
Normal rat	54	7	37.5	1.3	0.2
Avitaminous mouse No. 140	6	23	71	0	0
Normal mouse	60	21.5	17	1.4	0.1

This evidence of direct action of vitamin deficiency on the blood-forming tissue was confirmed by the striking changes recorded by Dr. Findlay (1921) in connection with the action of vitamin C on the bone-marrow. The marrow changes showed a replacement of the active blood-forming erythroblasts and polymorphic leucoblasts of the red marrow by a pale gelatinous aplastic tissue. Reduction of blood platelets has been reported not only in connection with vitamin A (see page 93) but as a more or less constant feature of the blood picture in avitaminosis. Experiments by Sherif and Baum (1927) showed a difference between human beings and animals in this respect.

Deficiency of vitamin A caused thrombocytopenia in 6 rats. In rachitic children the blood platelets were reduced in only half the cases studied. Keratomalacia also produced thrombocytopenia (2 cases). There was no direct change in the red blood picture. Deficiency of vitamin B caused a 25 to 50 per cent. reduction in number of platelets in 5 of 7 pigeons with experimental beri-beri. A similar change was not observed in 2 human cases. Vitamin C deficiency produced no change in platelets in either experimental or clinical cases.

(e) **CHANGES IN BONY TISSUE.**—The costochondral junctions of the ribs under deprivation of vitamin C and vitamin A, both together and separately, had been shown by Frances M. Tozer (1920) to develop characteristic lesions. Deficiency of vitamin C alone brought about an acute condition of swelling and fragility of the rib junctions with frequent fractures, and hæmorrhages about the fracture and in the marrow cavity and surrounding muscles; loosening of the teeth, and general illness of the animal. Microscopically the costochondral junction was found to be disorganised, the rows of cartilage cells had lost their alignment, the trabeculi were shortened, and the bone was frequently fractured on both sides of the junction, giving an appearance suggesting that the cartilage had telescoped into the marrow cavity.

The effects of deprivation of vitamin A alone were less characteristic. Fractures appeared only after 50 days, and there were no hæmorrhages comparable to those seen in scurvy. Microscopically the ribs showed slight progressive changes, with a tendency to disappearance of the rows of cartilage cells and trabeculæ, and atrophy of the marrow ingrowth of connective tissue, the cartilage often ending in a thin ossified band.

Lack of both vitamin C and vitamin A did not seem to hasten the onset of symptoms, but death usually intervened sooner than in C deficiency alone, and it was thought that lack

of vitamin A caused some atrophy of the marrow. It is noted that none of the guinea-pigs studied showed, after deprivation of vitamin A, the general pathological picture of rickets as seen in children, characterised by softening of bones and disorderly growth of the epiphysis, with the development of osteoid tissue.

(f) **DISTURBANCE OF LIVER FUNCTION.**—The effect of a vitamin-deficient diet on the function of the liver has been stated by Jono (1926) to be that of disturbance of glycolysis and oxidation of lactic acid. The livers were perfused with ox blood, to which, in some instances, glucose, or insulin, or both, were added. It was found that the sugar, lactic acid, and inorganic phosphorus of the perfused blood were all definitely more increased in the experiments with livers from the birds on the vitamin-deficient diet than in those with livers from the other birds. The addition of insulin to the blood hindered the increase of sugar and lactic acid, while the addition of sugar hindered the disappearance of sugar.

(g) **ANÆMIA.**—An anæmia produced experimentally by vitamin deficiencies has been reported by Funk, and more recently by Whitehead and Barlow (1929). According to the latter workers, vitamin B deficiency produces a more marked deficiency than vitamin A, but recovery is more rapid when vitamin B is administered. In their experiments, adult male rats, after attaining a basal weight level, were placed on special diets for a 10-week period. Eight different diets were tried, chiefly rice with various supplements, and bi-weekly observations, including body weight, erythrocyte counts, and hæmoglobin estimation were made. Polished rice diets, cooked and uncooked, resulted in a decrease in body weight and hæmoglobin level, the former proportionately greater than the latter.

Supplements of cod-liver oil as a source of vitamin A were of little or no benefit. The addition of 2 per cent. of compressed yeast resulted in loss of only 5 to 10 per cent. body weight and of only $3\frac{1}{2}$ per cent. in blood count with a parallel hæmoglobin figure.

Comparisons of diets were made by substituting lean beef for rice, adding 2 per cent. rice, substituting liver for rice and cod-liver oil, substituting liver for cod-liver oil alone, and the addition of yeast. These substitutions resulted in a more complete remission of the anæmia observed, and the greatest effect was produced by the lean beef muscle followed in order by liver, yeast, liver added to rice and yeast, yeast added to rice, copper, and iron, and yeast added to rice and iron. Weight recovery was complete in 4 weeks.

An anæmia of pregnancy, common in India, has been attributed by Wills and Mehta (1930) to vitamin deficiency. They point out that the disease is not essentially an anæmia of pregnancy but widely affects women throughout the population. It is only exacerbated by the exigency of pregnancy. The prevalence of the condition, together with the prevalence of inadequate diet, especially as regards its vitamin content, suggests an association between the two.

Keefer and Yang (1929) found no significant degree of anæmia in vitamin A, B, or D deficiency, except in cases of keratomalacia where infections (Kala azar, broncho-pneumonia, pulmonary tuberculosis) were also present. In scurvy, if the disease is of long duration and complicated by hæmorrhage, infection, or general undernutrition, anæmia may be present and responds quickly to antiscorbutic treatment. They point out that it is seldom that there is a single vitamin deficiency in man, though one type may predominate. It appears that a vitamin deficiency may lead to tissue changes which predispose to infection and thus lead to anæmia. This is particularly so when a condition such as dysentery arises and still further complicates the nutritional trouble.

(h) **TUMOUR GROWTH.**—McCarrison (1916) had observed in pigeons that diets deficient in vitamins favoured the action of an invisible virus capable of causing epithelial new-growth and of the disease known as epithelioma contagiosum. He points out the similarity of the epithelial hyperplasia produced by this virus to the downgrowths of epithelium figured in

some of Pappenheimer's (1924) gastric lesions in rats and comparable to the downgrowths of epithelium, resembling commencing carcinomatous change, found in one of his monkeys.

Fujimaki's observations (1927) on the occurrence of epithelial changes in vitamin A deficiency may also be read in conjunction with the above experiments. (See p. 91.)

Funk (1914, 1915) believed that cancer is probably a dietetic problem. If vitamin B is deficient or absent in the diet of an animal with a Rous chicken sarcoma, the growth of the tumour slackens and grafts are not successful, a result which is also seen in the tumours of rats and mice. Tumours of one species cannot be normally grafted on to another species, but by feeding rats on mice tumours for 10 days a series of successful grafts can be made through several generations, if fed on the same diet. The substance in the tumour ("trophone") responsible for this effect resembles the vitamins in its mode of action.

Funk (1919) claims to have isolated several crystalline substances from commercial insulin, one of which (A) reduces the blood-sugar in diabetics but is almost without effect in the normal person, whilst another (B), taken by mouth, increases the blood-sugar to the point of glycosuria.

He states that this substance B stimulates the growth of tumours while the substance A has the opposite effect. He believes that insulin, containing this factor B₁, is found in all flesh foods.

On these grounds Funk suggests that the great decrease of cancer during the war was due to the decreased consumption of meat, and therefore of this substance (B). In attempting to explain why, if flesh diets predispose to diabetes and cancer, carnivorous animals and the flesh-eating Eskimos should not be prone to these affections, he suggests that they have acquired some form of immunity to the harmful substance in their diet.

Saiki (1927) has described a carcinoma of the stomach developing in animals fed on vitamin-deficient diets, and Erdmann (1927) and his co-workers, though unable to confirm the occurrence of these carcinomatous changes in the stomach, found adeno-carcinoma of the breast in 2 out of 80 rats kept for 5 months on a diet in which supplies of vitamins were given during one week out of every three. They state that the rats had all been obtained from a known stock, and spontaneous tumours had never been found to arise among many hundreds kept for other purposes, and that confirmation of Saiki's results was not expected since their results were published at a time when animals had been dieted for only half the time reported by Saiki to be necessary for the development of carcinomatous changes in the stomach.

Erdmann and Haagen (1928) regard cancer as the result of combined endogenous and exogenous factors, the former represented by the effects of vitamin imbalance, either general, or resulting from local changes; the latter by the miscellaneous external irritants known to be factors in the production of cancer. Neither of these two factors can be present in too great intensity; a "subthreshold" relation on the part of both is necessary.

In their later experiments (1928) 80 rats were kept for considerable periods on a diet free from vitamins, except vitamins B and D, which were supplied in excess by brewers' yeast. Of these, 4 developed tumours, while a large number of rats of the same stock, on a normal diet, did not develop any. The greater occurrence of tumours in rats with abnormal vitamin supply, with excess of vitamin B, is interpreted as due to an increased predisposition to tumours resulting from that abnormality, with a possible additional factor of hereditary sensibility to alterations of vitamin. As a result of vitamin alteration, there results a freeing of normal cell bonds, so that they hypertrophy freely in response to local irritation, eventually forming malignant tumours.

With regard to inoculable tumours the evidence for any influence of avitaminosis is quite inconclusive.

Thies (1929) states that in mice fed on a vitamin-free diet for 10 days before inocula-

tion with tumour, and for a similar period following the inoculation, there were no apparent differences from normal mice in respect to frequency of takes, nor in survival after successful inoculation. Similar more or less negative evidence will be discussed under "Vitamin B."

(i) **ENDOCRINE DISTURBANCE.**—The chief work in this connection has been done by McCarrison in his experiments on different animals with different classes of dietary deficiencies. The diets used were as follows: (1) Deficient in all three classes of vitamins and in suitable protein, but rich in carbohydrates; (2) deficient in B and C vitamins, and rich in carbohydrates and fats; (3) deficient in B vitamin, but rich in carbohydrates and fats; (4) deficient in A and B vitamins; (5) B vitamin only lacking; (6) C vitamin deficient. He attributed the effects of these dietaries on the endocrine organs to the action in varying combination of, firstly, deficiency of vitamins; secondly, imperfect balance of the proximate principles of the food; and, thirdly, the chance occurrence of pathogenic agents in the body. As the result of dietetic deficiencies, all endocrine organs except the adrenals and pituitary body, which underwent enlargement, suffered varying degrees of atrophy and lowering in functional capacity.

The effects, such as atrophy and hæmorrhagic enlargement, differed according to the species of animal, the character of the deficient diet, and the particular organ, not all the animals responding in the same degree when fed on the same diet. The adrenal glands, the most susceptible of all the endocrine organs to dietetic defects, were found to become enlarged as the result of all six classes of deficient dietaries and also in consequence of starvation. But the character of the enlargement varied according to the class of diet, both histologically and as regards adrenalin content—*e.g.* a scorbutic diet in guinea-pigs produced hæmorrhagic infiltration of the cortex, associated with a great reduction of the adrenalin content, whereas in pigeons autoclaved rice and butter gave rise to marked hæmorrhagic infiltration of the medulla and, as a rule, a low adrenalin content. With food deficient in vitamins and suitable protein and rich in carbohydrate, but well balanced with regard to proximate principles, the enlargement of the adrenals was less marked and the adrenalin content lower than when this balance was wanting, in the case of pigeons and monkeys. The quality of the food, therefore, governed the amount of adrenalin content, as also did concurrent infections. Œdema, usually hydropericardium, was often associated with enlargement of the adrenals, but did not occur in the absence of a corresponding increase of adrenalin content. It was found that the addition of fresh butter to a dietary of autoclaved rice lowered the incidence of œdema in pigeons, and appeared to afford complete protection up to the thirty-sixth day of the experiment, after which, however, the butter gave no protection against the occurrence of *wet* polyneuritis, although some of the pigeons, 19 out of 29, had already developed *dry* polyneuritis.

The lowered incidence of œdema was associated with a lowered adrenalin content, yet an increase in weight of the glands occurred. The limitation of protection against *wet* polyneuritis to 36 days was attributed to the development of pathological changes in the digestive organs resulting from deficient dietaries and possibly from an inhibition of the assimilation of substances on which protection against œdema was dependent. Butter was also found to vary in its capacity to protect against œdema in pigeons fed on autoclaved rice, due probably to a difference in the lipochrome content of the butter, according to the particular fodder on which the cows providing the milk for the butter were fed. It was therefore suggested that the hypothetical "anti-œdema" substance might be of the nature of lipochromes.

Stammers (1926) found slight hypertrophy of the adrenals with diminished adrenalin content in cattle which showed œdema due to "defective nutrition," while in rats on diets deficient in vitamins A, B, and C, dying at intervals of 8 to 97 days, there was no adrenal hypertrophy. Changes in thyroid, parathyroid, and pituitary have been studied chiefly in connection with vitamin B deficiency, and will be discussed in that special relationship.

Tsuki's hypothesis (1921) that general vitamin deficiency symptoms are a consequence of hypo-function of the thyroid has not received much support. The effects of avitaminosis on the sexual glands are also specially related to deficiency of vitamin B and vitamin E.

On the basis of extensive experiments with rats, Evans (1928) concludes that when the rest of the ration is adequate, neither acute nor chronic deficiency of the antineuritic vitamin B will result in anatomical or functional change in the male germinal epithelium. This is of considerable interest in view of the fact that the indispensability of vitamin E for normal reproduction in both the male and the female has been demonstrated. Evans (1928) has carried out further studies bearing on the specificity of the antisterility factor E. It was shown in this investigation that a potent source of vitamin E in the diet exerts a stimulating effect on the growth of both male and female rats, which effect is brought about quite apart from the influence on the sex glands. The acceleration in growth was observed in castrated as well as in intact males. While these studies indicate that the function of the antisterility vitamin may be more diverse than heretofore believed, they tend to show that its action is also less specific. It is suggested by Cotte and Carcassonne (1927) that a diet deficient in any of the vitamins, especially in young girls, may cause irreparable damage to the ovary. They consider that vitamins play the rôle of true hormones, constituting stimulants to ovarian activity, and preventing the occurrence of both amenorrhœa and sterility.

(j) **DEFECTIVE TEETH.**—The relation of vitamin deficiency to defective teeth has been investigated by Edward Mellanby (1918 onwards), and the results of their experiments prove that lack of the fat-soluble vitamins, combined with an excess of cereal in the diet, produces imperfect formation of the teeth in children. Other investigations, emphasising the influence of diet on the teeth, have been carried out by McCollum, Simmonds, Kinney, and Grieves (1922). Changes in the teeth of rats were produced after feeding on diets with various defects, such as deficiency of the antirachitic vitamin, or unbalanced quantities of calcium and phosphorus. They claim to have produced caries-like lesions in the teeth of rats by these means.

More recent experiments by Mellanby (1928) have tended to focus the vitamin deficiency responsible for caries of teeth specifically upon the antirachitic vitamin, and the question will be further discussed under that heading. (See p. 186.)

(k) **SUSCEPTIBILITY TO INFECTION.**—A lowered resistance to infection has been specifically investigated with regard to deficiency of vitamins A and B, and will be more fully dealt with under the heading of each of the vitamins. (See p. 97 *seq.*, and p. 275.)

1. **Effect of Deficiency of Vitamins A and B.**—McCarrison's experiments (1923) on the occurrence of ophthalmia in pigeons fed on parboiled rice have brought an interesting view of the relationship of vitamins A and B in deficient diets and their respective powers of protection against bacterial invasion. He found that parboiling rice destroyed such fat-soluble vitamin as rice contains; the development of ophthalmia was favoured by the presence in the parboiled rice of just sufficient vitamin B to keep the birds alive—on the borderland of insufficiency—for periods sufficiently long to enable the deficiency of vitamin A to exercise its well-known effect in producing ophthalmia; it was due in short to a peculiar combination of dietary defects relative both to vitamin A and to vitamin B—defects which ultimately inhibited the protective resources of the conjunctiva and of the eye against invasion by microbes which in health are harmless to either.

McCarrison's experiments on the gastro-intestinal tract in monkeys (1922) have shown that vitamin deficiency resulted in its greatly increased susceptibility to attacks by bacterial and other pathogenic agents. The gastro-intestinal changes showed clearly how vitamin deficiency, quite apart from its action in disturbing nutrition and assimilation, could be a great causal factor in disease—(a) by producing or by favouring the production of definite

pathological conditions of catarrh, congestion, inflammation, ulceration, and other changes in the intestinal tract, especially mucous colitis; or (b) by producing marked changes in the blood-forming tissues, namely, atrophy of lymphoid tissue in the intestinal mucosa (vitamin B), or, as shown by Dr. Findlay (1921), equally pronounced aplastic changes in the blood-forming bone-marrow (vitamin C).

Cramer's (1922, 1923, 1924) results on withholding vitamin B supplemented those of McCarrison by showing how lowered resistance was clinically manifested by fall of temperature, loss of appetite, constipation, a delayed passage of food from the intestinal tract, a progressive atrophy of the lymphoid tissue which manifests itself by a progressive lymphopenia and impaired absorption of food.

In the effects of vitamin A deficiency the conditions are different. The animals develop bacterial infections spontaneously, even when their general nutrition is not seriously impaired and when their temperature has not fallen below normal.

A deficiency disease due to a combined lack of vitamins A and B has been described by Wright (1927, 1928) in Sierra Leone, where the staple food of the people is rice and cassava, eaten with palm oil and dried fish, neither of which, when added up to 10 per cent., can compensate for the vitamin A deficiency in the staple foods. Symptoms of B avitaminosis can easily be precipitated in the people of this district. The deficiency naturally affects pregnant women and growing children particularly, and is best studied in pregnant women owing to the rapid progress of the disease in these cases. This combined A and B avitaminosis gives rise to a definite train of symptoms of which the earliest are various degrees of glazing of the tongue accompanied sometimes by soreness. The angles of the mouth become sore (with a thrush-like appearance), the tongue is tremulous (the first sign of B deficiency), the eyes show changes, often with conjunctivitis. If the condition progresses, other nervous symptoms arise. Changes in the knee jerks, paræsthesias of the limbs, and dimness of vision may occur. Cases precipitated by pregnancy usually show symptoms after delivery, and the nervous symptoms may be so pronounced that walking is impossible. Treatment consisted of cod-liver oil and marmite, and, as a rule, recovery occurred without any local treatment. The nervous symptoms take much longer than the mucous membrane lesions to recover. The sight is usually the last to recover if it has been severely involved, but ultimately it responds to dietetic treatment.

Stannus (1930) suggested that the conditions described by Wright may have a relationship to pellagra, or, at any rate, to a syndrome produced by vitamin B₂ deficiency.

2. Non-specific Lowering of General Resistance.—The experiments of Webster and Pritchett (1924), on the effect of diet on host resistance, which they themselves at that time regarded as not altogether conclusive, were considered to indicate a decreased general resistance of the host, largely non-specific in nature, due to a diet lacking in vitamins. In these experiments three varieties of resistance were investigated:

(1) **RESISTANCE TO MOUSE-TYPHOID INFECTION.**—The diets compared were a control diet of bakers' bread soaked in milk, supplemented by weekly feedings of oatmeal, buckwheat, and dog biscuit, and the "McCollum diet," consisting of wheat, casein, milk powder, butter fat, and inorganic salts.

The results of these experiments show that mice fed on the McCollum diet are more resistant to mouse-typhoid infection than are similar mice nourished on a bread-and-milk diet.

(2) **RESISTANCE TO BICHLORIDE OF MERCURY.**—The McCollum resistance curve to a 1:200 dilution of bichloride of mercury was much more gradual, and the final mortality rate 15 per cent. below that of the mice on the control diet.

(3) **RESISTANCE TO BOTULINUS TOXIN.**—The curves indicate that the resistance on the McCollum diet is greater, but the final mortality is the same for each diet.

The later experiments, in 1925 and 1926, seem to have been rendered slightly indefinite by the fact that the periods over which they were observed were not the same in all cases. There would appear, however, to be little doubt that the data afford definite support to the conclusion that the addition of butter fat, or of cod-liver oil, to the diet increases the resistance of mice to typhoid infection, or, in other words, decreases the mortality from it. With regard to the existence of clear-cut seasonal variations in resistance, or their modification by dietary factors, the conclusions can scarcely be said to have been definitely established.

Webster and Pritchett's own conclusions on this point are as follows: "While seasonal fluctuations in resistance to mouse-typhoid were not completely eliminated by the various modified diets, they were nevertheless reduced, the modified diets tending to stabilise the death-rate at a point lower than that usually reached by the mice on the control diet."

3. Resistance to Diphtheria Toxin.—The reactions of avitaminous animals to diphtheria toxin have also been studied by Werkman and his co-workers (1923, 1924), and they have concluded that rats suffering from the lack of vitamins A or B succumb to the effects of smaller injections of diphtheria toxin than do normal animals on a complete diet. The susceptibility of the avitaminic animal is not due to any rupture of the immunity mechanism that normally serves to prevent an injurious action by diphtheria toxin. The ability of the animals to produce antitoxin is not disturbed, and a small production of between 0.5 and 1.0 unit per c.c. occurs after about five sublethal injections of toxin. The absorption of diphtheria toxin by the cells of B-deficient rats is not greater than occurs in normal animals, and the toxin is found floating unattached for hours in the serum after injection.

The injection of diphtheria toxin into the rat produces a marked and sudden drop in blood pressure, usually occurring on the second or third day after the injection is made. If a fatal dose of toxin has been injected, the drop is progressive to the point of death. The rat tolerates markedly low blood pressures and body temperatures several days before death intervenes.

4. Effect on Tuberculosis.—That there is a definite relation between a defective supply of vitamins and the incidence of tuberculosis is suggested, chiefly on the basis of clinical experience, but experimental evidence is not entirely conclusive.

Japanese investigators have recently attempted to trace the connection between tuberculosis and the active principle in cod-liver oil.

Hattori and Matsiura (1928) of the Nagoya Sanatorium suggest that vitamin A is one of the most favourable remedies for use in tuberculosis, but that chemically pure preparations are not as good a remedy as a cruder preparation. At the Institute for Physical and Chemical Studies, anti-xerophthalmic vitamin A was found to have only indifferent effect against tuberculosis, but xerophthalmia was amenable to the chemically pure preparation of vitamin A. Vitamin B and vitamin C deficiency affected tuberculosis unfavourably.

Their results would seem to confirm those of Smith, McClosky, and Hendrick (1926), who have stated that rats inoculated with the tubercle bacillus and fed on a complete diet were highly resistant to subsequent injection of tuberculin, whereas similar rats kept on a diet deficient in fat-soluble vitamin were peculiarly sensitive to such injections.

Hagedorn (1929) states that, in rats on a diet containing insufficient vitamins, the generalisation of inoculation tuberculosis occurs earlier, and the resulting lesions are more extensive than they are in rats on a diet containing sufficient vitamins. The effects of vitamin A deficiency and of vitamin C deficiency are approximately the same, but the effects of vitamin B deficiency are less marked.

Zilva and Schutz (1927), however, have found it difficult to come to any definite con-

clusion. In their summary they state that they found some evidence for saying that "a large excess of fat-soluble vitamins in the diet, as supplied by cod-liver oil, inhibits the formation in rats of these tuberculous tumours, but such evidence was by no means conclusive."

A fairly extensive investigation into the results of giving a diet rich in vitamins in cases of advanced tuberculosis has recently been carried out by Mayer and Kugelmass (1929). The diet had a caloric intake of about 50 calories per kilogram of body weight, and many of the calories were furnished by vitamin-bearing fats. The daily protein intake was 92 grms., the carbohydrate 378 grms., and the fat 174 grms., and a large proportion of the dietary consisted of raw food, while that which was cooked was abundant in all the vitamins. The results, after 6 months of this dietary treatment, were on the whole favourable, 8 patients out of the 20 showing definite clearing of the lungs by physical and Röntgen examination, while a diminution in fatigue, pains in the chest, and alimentary disturbances were conspicuous. Mayer and Kugelmass themselves, however, point out the fallacy of attributing these results entirely to the dietary régime; the psychic element, the enforced rest, the occasional tendency of the disease to subside spontaneously, the wholesome food, its scrupulous preparation, and careful cooking, and the individual service are factors that must be considered in a final analysis.

The beneficial effect of treatment of infections, especially tuberculosis, with a combination of vitamins is emphasised by some recent experiments of Pfannenstiel and Scharlau (1930). They found that in medication with only one vitamin the therapeutic effects were negligible, whereas the simultaneous administration of viosterol and of dry yeast had a curative effect. They explain this effect by suggesting that the vitamins have different therapeutic effects, *e.g.* while vitamin D and calcium counteract inflammation, vitamins B and A hasten the process of recovery. More recent experiments have attempted to establish a connection between vitamins A and D and the incidence of tuberculosis, and will be discussed under the separate headings of vitamin A and vitamin D.

5. Susceptibility to Certain Poisons.—The influence of vitamin deficiencies on susceptibility to certain poisons has received different emphasis from the results of experiments by different workers.

In support of the hypothesis that in vitamin-deficient animals there is a lessened resistance to infection. Gros (1921) brought forward experimental evidence that there is in such animals also a hypersensitiveness to arsenical compounds. Subcutaneous injections of 1 per cent. solutions of various arsenical compounds into white rats which had developed rickets and xerophthalmia as a result of a lack of vitamins, and control injections in normal rats, showed that the toxicity of arsenious acid, neosalvarsan, atoxyl, and arsacetin was much greater, and the fatal dose much smaller in the vitamin-poor animals than in the normal animals. The difference with arsacetin was particularly striking.

Smith, McClosky, and Hendrick (1926) found increased susceptibility to pilocarpine and to ergotoxin in rats deprived of vitamin B, while rats fed on a diet deficient in fat-soluble vitamin showed a much lowered resistance to ergotoxin and to morphine; these animals also showed a slightly increased susceptibility to histamine, strophanthin, strychnine, atropine, cocaine, and apocodeine.

These results were interpreted to mean that the increased susceptibility to ergotoxin was due to altered functioning of the autonomic nervous system.

Van Leeuwen and Verzar (1921), on the other hand, investigating the part played by diminished sensitiveness of smooth musculature in the causation of the severe symptoms of avitaminous animals, found that these animals did not react differently from normal animals to the action of adrenalin, histamine, choline, atropine, and pilocarpine.

From the facts that the function of muscle, both striated and smooth, is damaged in

avitaminous animals and that the sensitivity of the smooth muscle of such animals to drugs is unchanged, they argue that it is probable that the decreased activity of smooth muscle in avitaminosis is due to the absence from the body of such animals of the normal stimulating chemical agents. This view agrees with the conception of Abderhalden, Bang, Uhlman, and others, who consider that the vitamins have a stimulating action on smooth muscle similar to that of pilocarpine.

6. Immunity Reactions.—Werkman's experiments (1923) showed no differences in the antibody production of animals deficient in vitamin A and that of the controls, and the deficient animals elaborated agglutinins, precipitins, hæmolysins, and bacteriolysins; in other words, the immunity mechanism of these animals is just as capable of resistance to most experimental infections as that of normal animals.

Zilva (1919) has also investigated the influence of deficient nutrition, on the production of agglutinins, complement, and amboceptor. He used rats and guinea-pigs, and studied deficiencies of the elements, Ca, Fe, K, Cl, P, and Na, of certain amino-acids, and of the antiscorbutic, antineuritic, and fat-soluble vitamins; restriction of total calories was also studied. It was found that a deficiency of phosphorus was the only one which had any effect in lowering the titre of agglutinins and amboceptor.

XII. QUANTITATIVE ESTIMATION OF VITAMINS.

The question of the determination of the actual quantity of vitamins in certain food-stuffs could be approached until recently only from the biological aspect, and workers in France, such as Randoin and Simonnet, Lesné and Clément, still assert that the biological method is the only rational method. Lesné and Clément (1928) insist that the physiological or therapeutic activity of vitamins is only comparable to that of endocrine secretions, and that, in order to appreciate the richness in vitamins of a certain substance, it is necessary to have recourse to its experimental activity on animals. Their definition of the physiological unit of a vitamin is "the daily dose necessary, and sufficient to protect an animal of known weight receiving a diet deficient in the corresponding vitamin." They believe, however, that the biological method is only practicable for determining the actual vitamin value of any substance, that it is not possible to establish a definite relationship between the units determined by animal experiment and the dosage which it is necessary to prescribe for growing children, since the child is more susceptible to lack of vitamins and is rarely submitted to a total deficiency. Their requirements of standardisation include both the time and condition of preservation of the vitamin-containing substances, on account of the injurious effects of heat, desiccation, oxidation, exposure to sunlight, and age. They require, therefore, that every vitamin-containing substance should be accompanied by a notice stating not only the vitamin content, expressed in physiological units, but the date of its manufacture, and the latest date when it may satisfactorily be used.

Randoin and Simonnet (1927) describe the biological method of estimation under two headings:

(a) **THE CURATIVE**—depending on the power of the vitamin-containing substance to suppress the disturbances caused by the administration of a deficient diet. This method is quicker, but less exact than the second, or preventive. Its fallacies lie in the possibility of—(1) secondary disturbances, curable by other agents than the causal one, or incurable by it; (2) a mixed deficiency, in which the quantitative or qualitative requirements of other substances may be different in the presence or absence of the substance in question.

(b) **THE PREVENTIVE**—in which disturbances known to be due to certain deficiency are prevented by giving foods containing the deficient element. In order to obtain exact results by this method, the diet must be deficient only in the particular element investigated.

(c) **DIETS USED IN QUANTITATIVE ESTIMATION.**—With regard to the diets used in the biological assay of vitamins it is interesting to note that Drummond (1926) believes many of the discrepancies and contradictions of results in modern vitamin research to arise from failure to use adequately purified diets. In his own experiments he has recently decided to eliminate fats entirely, since it is in the purification of fats, the nature of whose unsaponifiable portion is practically unknown, that the greatest difficulty is encountered, and he includes vitamins in the form of concentrates in minimum quantity.

The following is an example of the constitution of the diets which Drummond uses in testing for the presence of vitamins :—

Casein (purified as far as possible)	.	.	15.0
A.R. Sucrose	.	.	8.3
Salts consisting of	$\left\{ \begin{array}{l} \text{CaCO}_3 \text{ 50} \\ \text{NaCl 25} \\ \text{Na}_2\text{HPO}_4 \text{ 15} \\ \text{K}_2\text{HPO}_4 \text{ 10} \end{array} \right\} 2.0$		
Concentrates	$\left\{ \begin{array}{l} \text{A} = 0.01 \text{ mgrm.} \\ \text{B}_1 = 0.0026 \text{ grm.} \\ \text{B}_2 = 0.04 \text{ grm.} \\ \text{C (unnecessary for growth of rat)} \\ \text{D} = 0.0001 \text{ mgrm.} \\ \text{E} = 0.02 \text{ mgrm.} \end{array} \right.$		
(Total weight 42.6 mgrms.)			

Growth on such a diet proceeds at the rate of an average increase of 6.3 grms. per week, as compared with a normal increase of 13.7 grms. Drummond himself has pointed out that a fallacy in experiments with such strictly purified diets lies in the fact that the animals refuse to take more than the minimum required to keep them alive.

(d) **ANIMAL SPECIES IN VITAMIN ASSAY.**—Since animals differ in their vitamin requirements it is necessary to have some form of standardisation of the species used in order to avoid unnecessary divergence of opinion.

For the investigation of vitamins A, D, and E, and the growth factor of vitamin B, the rat has been found most suitable; for the antineuritic factor of vitamin B and for what the French workers, particularly Madame Randoin, regard as the “nutritive” factor (corresponding more or less to the growth factor described by Seidell), the pigeon is most generally employed; while the guinea-pig and the young dog are found particularly sensitive to vitamin C deficiency.

(e) **UNITS OF BIOLOGICAL ESTIMATION OF VITAMINS.**—The physiological units obtained by biological experiments carried out, as above, have been worked out by Lesné and Clément (1928) as follows :

Vitamin A—the daily dose sufficient and necessary to protect a young rat against xerophthalmia and cachexia, and to allow development such as occurs in animals on full diet.

Vitamin B—the daily dose sufficient to prevent the occurrence of beri-beri in a pigeon of 300 to 350 grms. on a diet of polished rice. Dried brewer’s yeast contain less than 10 physiological units per gram of vitamin B.

Vitamin C—the daily dose necessary and sufficient to protect an animal on a diet deficient in vitamin C from scurvy and death. Lemon juice contains on the average less than 1 to 2 physiological units.

Vitamin D—the daily dose necessary to protect a young animal against rickets. Cholesterol, fresh and freshly irradiated, contains 33 physiological units per gram. Ergosterol, irradiated (according to Hess and Windaus) may contain 500,000 physiological units per gram.

XIII. QUANTITATIVE ESTIMATION OF VITAMIN A.

(a) **BIOLOGICAL METHOD.**—A standard cod-liver oil should possess at least 45 physiological units of vitamin A per cubic centimetre; that is to say, 1 drop per day should protect a rat on a diet deficient in vitamin A. Oils rich in A may contain 70 to 100 physiological units per cubic centimetre.

In recent experiments (1929) Drummond and Morton have used ethyl oleate, specially prepared by saponification of olive oil, and the removal of all unsaponifiable matter, as a useful solvent for vitamin A. This preparation must be kept *in vacuo* or in the dark to prevent deterioration. They found that more consistent values were obtained by comparing the dosage necessary to give 3 grms. of growth per week than by comparing the rates of growth on the same dosage.

FACTORS AFFECTING THE ACCURACY OF THE BIOLOGICAL TEST FOR VITAMIN A.

(1) **Adequate Supply of Vitamin D.**—Chick (1926) has pointed out a source of error in the variable amount of reserves of fat-soluble vitamins present in young stock rats when placed upon diets deprived of fat-soluble vitamins.

Steenbock and his co-workers (1923, 1924, 1925, 1927) maintain that vitamin A can only be estimated approximately when the experimental animal is provided with an adequate supply of vitamin D, as *e.g.* by irradiation with ultra-violet light. They have therefore recommended a special method of determination of vitamin A. Young rats placed upon diet deficient in fat-soluble vitamins are irradiated daily by a mercury-vapour quartz lamp. The degree to which growth is maintained after addition of various foodstuffs is a measure of their vitamin A content.

Drummond, Coward, and Handy (1925) have described a similar method in which vitamin D is supplied in the form of irradiated crystals of pure cholesterol, while Steenbock and Coward (1927) have also recommended irradiating the basal synthetic ration.

A quantitative differentiation of vitamins A and D has been suggested by Sherman and Hessler (1927). They provide vitamin D either by irradiation, or by keeping down the amount of growth due to vitamin A, so that vitamin D is stored. The animals are kept from 4 weeks old on a diet deficient in vitamin A until growth has ceased, so that the stores of vitamin A are depleted. Then the daily allowance of vitamin A, containing substance necessary to support a gain of 3 grms. per week, is equivalent to 1 unit of vitamin A.

(2) **Length of the Testing Period.**—The accuracy of the quantitative determination of vitamin A may be affected by the length of the testing period.

Coward and Key (1928) noted in the course of vitamin A assays that animals apparently in the same condition would respond to equal doses of vitamin A either by—(1) immediate resumption of rapid growth; (2) immediate resumption of sub-normal growth; or (3) a long latent period, often of several weeks, followed by a resumption of normal growth. It is suggested that, in the last case, growth is suspended until possible pathological conditions are cleared up, but this is not the complete explanation, for more uniform results are obtained when rats from one litter are used. They suggest that a long test period of 8 or more weeks should be used, and Sherman and Burtis (1928) support their suggestion since they found that shortening the test period from 8 to 5 weeks resulted in greater average differences and gave the material tested a higher numerical rating in vitamin A value. They consider that the most reliable method for the quantitative estimation of vitamin A by means of comparing growth rates in rats is to adopt a rate of gain of 3 grms. per week as a standard after the period of depletion. With this rate of gain per week males and females may both be used in comparative tests, but if the level of vitamin A intake is increased, a normal rate of growth is

approached, and at the age of the test period the normal growth curve of the male is steeper than that of the female.

(3) **Constant Source of Vitamin A.**—Bezssonoff (1929) has pointed out that one of the chief difficulties of vitamin A assay lies in obtaining a uniform and constant source of vitamin A just sufficient to ensure life and reproduction. He suggests the use of oats (90 per cent.), which, according to Steenbock and Coward (1927), is practically devoid of vitamin A, with the addition of bran (8 per cent.) and cod-liver oil (0·2 per cent.). On this diet mice reproduced normally, and their young also showed themselves fertile.

(4) **Variations in the Breeding Diet.**—According to Gudjónsson (1930) the incidence of symptoms of vitamin A deficiency is affected by the amount of vitamin A in the mother's diet. He states that the "fore-period," *i.e.* the period from the time the 30 days' old rat is fed on a vitamin A-deficient diet until it shows definite signs of A-avitaminosis is definitely increased by increasing the amount of vitamin A in the mother's diet. Growth in the after-period (period when the vitamin A supplement included in the diet—8 to 10 weeks in author's work) is dependent on that in the fore-period, and thus weight curves for the after-period are only comparable when the fore-periods have been of equal length and the weight increase about the same.

In order to eliminate variations in the breeding diet, Gudjónsson uses the following: Skimmed milk powder 30, rice flour 40, yeast (autolysed without drying) 15, coco-nut oil with shark oil (0·3 per cent.) 15. The litters obtained on this diet have a constant and short fore-period (about 4 weeks). With this diet he thinks that the preventive method may be used as successfully as the curative. He includes neither vitamins D nor C in his vitamin A-free basal diet, and, provided the prenatal feeding is uniform, finds that the lack of vitamin D does not alter the result of vitamin A assays.

(5) **Supply of Vitamin B Complex in the Basal Diet.**—Studies by Honeywell, Dutcher, and Ely (1929) indicate that the technique of vitamin A assay is influenced by the yeast used as a source of vitamin B complex. Using different samples of yeast, they found that some contained appreciable amounts of the anti-xerophthalmic, others of the growth-promoting factor. They obtained differences in the curative response on 2 drops of butter fat, which varied from 100 to 300 per cent., depending on the kind and amount of yeast ingested. They conclude that comparative quantitative determinations of vitamin A in foods are not possible unless each new supply of yeast is tested and the technique standardised.

(b) **CHEMICAL TESTS.**—In England the biological test is regarded as unsatisfactory, compared with chemical tests, where such can be instituted. Drummond and Watson's first indications of the possibility of such a test for vitamin A in cod-liver oil was welcomed in 1922.

Drummond and Watson examined a series of fish oils and found that their vitamin content varied about tenfold, and that the intensity of the colour reaction also varied greatly, and they showed that the variations in the vitamin content and in the intensity of the colour reaction were roughly parallel. They also showed that the passage of air through cod-liver oil at 100° C. slowly destroyed the vitamin A present, that the colour reaction was abolished by the same process, and that the vitamin and the chromogenic substance disappeared at the same rate. They conclude that: "The few properties of the (chromogenic) substance which are known, as well as the available data regarding its distribution in natural products, show certain resemblances to the unidentified dietary product known as vitamin A; and, without assuming the identity of the two factors, it is suggested that the association may be of some significance."

The validity of the colour reactions was tested by a series of experiments organised by the Accessory Food Factors Committee at the request of Dr. H. H. Dale, Chairman of the Second International Conference (League of Nations) on the Biological Standardisation of

Certain Remedies, Geneva, August to September 1925. A sub-committee of this Conference (Dr. H. H. Dale, England ; Prof. Poulsson, Norway ; Prof. Voegtlin, United States) had been appointed to arrange for a special test to be made of the validity of the colorimetric method for estimation of vitamin A described by Rosenheim and Drummond in comparison with the biological method.

The test will be described in detail under the separate heading "Vitamin A," but the general results, while leading to the conclusion that the colorimetric method afforded information consistent with that derived from the biological tests, showed that the biological method "does not, at present, permit of sufficient accuracy for the presentation of the results in numerical form." Another investigation, on the same lines, by Clare and Soames (1928) was considered to have demonstrated how small is the degree of accuracy attainable with the present biological methods unless a prohibitive number of animals is used, and that the biological method of testing (U.S. Pharmacopœia) by vitamin A assay only is open to criticism.

On the other hand, an investigation by Schmidt-Nielsen (1929) into the vitamin A content of fish oils, led him to the conclusion that the results obtained by the two methods were not consistent. A repetition of Schmidt-Nielsen's experiments by Ahmad and Drummond (1930), however, have not confirmed this conclusion.

Ahmad and Drummond, using six samples of fish-body and fish-liver oils, including two samples tested by Schmidt-Nielsen himself, found that the results of animal tests agreed reasonably well with those of the colorimetric (SbCl_3) method. They suggest that the disagreement between Schmidt-Nielsen's results and their own may be due to differences in technique and to the fact that he expressed his results in U.S. pharmacopœia units. This latter is especially liable to lead to erroneous results when oils of low vitamin A content are examined.

XIV. QUANTITATIVE ESTIMATION OF VITAMIN B_1 .

(a) **BIOLOGICAL METHOD.**—Comparative estimations of the amount of the antineuritic vitamin in a series of foodstuffs can be made by determining the minimum amount of each foodstuff which must be added to the diet to prevent the onset of polyneuritis.

A method of estimating the potency of antineuritic concentrates has been described by Smith (1930). He administers minimum curative doses of vitamin B_1 concentrates by intravenous injection to rats which have been kept on a diet capable of producing polyneuritis.

FACTORS AFFECTING THE ACCURACY OF BIOLOGICAL TESTS FOR VITAMIN B_1 .

(1) **Nature of the Preparatory Diet.**—Kucera (1928) maintains that the comparative estimation of vitamin B in foodstuffs is subject to considerable error if the birds are transferred directly from a normal diet to one of polished rice which, in addition to containing no antineuritic vitamin, is also deficient in proteins, fats, and possibly mineral substances. Thus, in determining the comparative values of a number of legumes and cereals, pigeons were, during the preparatory period, fed on polished rice to which had been added the particular foodstuff to be tested, the latter being freed from vitamin B by sterilisation. The minimum amount of the unsterilised foodstuff necessary to protect the birds from the onset of polyneuritis when added to this deficient diet, was then estimated. The results obtained for different kinds of cereals and legumes showed differences of 500 to 1000 per cent. over those previously obtained when the birds had been transferred directly from a normal diet to one of polished rice only.

Kinnersley, Peters, and Reader (1928) have also found that the previous feeding of the bird, before the experiment was commenced, influenced the curative dose required, and error could be largely avoided by previously feeding the birds on a standardised diet. Most birds

reacted constantly to rice feeding so that symptoms appeared on a constant day after the beginning of feeding ("day-constant," varying from 14 to 28 days), but no correlation could be found between the colour or weight of the bird at the onset of symptoms and this constant.

(2) **Amount of Protein in the Basal Diet.**—Funk (1916) stated that in pigeons the vitamin B requirement decreased as the protein intake increased, while Hartwell (1925) stated that rats required more vitamin B on a higher protein diet. Sherman and Gloy (1927), however, found that, using casein as the protein and orange juice as the source of vitamin B₁ diets containing no vitamin B, but otherwise adequate, and in which the amount of protein varied from 12.54 per cent., produced the same survival period, independent of the amount of protein in them. Neither did these protein variations affect the form of the weight curves, in groups of animals receiving different quantities (both maintenance and submaintenance allowances) of vitamin B.

(3) **Requirements of the Nursing Young.**—A quantitative method for estimating the vitamin B requirements of nursing young has been devised by Sure (1928). He claims that the method is sensitive to 1 mgrm. per nursing per day. A vitamin B unit, applied to nursing young, has been defined as the daily amount of vitamin B which must become available to a nursing albino rat weighing 30 grms. in order to permit a gain of 10 grms. in 7 to 10 days. One gram of a dehydrated baker's yeast, Federal brand, contains 7 units of vitamin B. Applying this quantitative biological method of assay to vitamin B concentrates, variations of 14.4 to 125 units per gram were found.

(4) **Sources of Extraordinary Cure.**—Investigations of Kinnersley, Peters, and Reader (1928) tend to show that certain essentials of technique must be fulfilled if the test for vitamin B₁ is to be accurate. Birds were found to be temporarily cured of acute polyneuritis by giving small doses (50 mgrms.) of glucose or by bringing them from the cold into a warm room. As a routine, therefore, birds are given a dose of glucose in water and left in a warm room for 2 to 3 hours in order to eliminate sources of extraordinary cure before testing with the vitamin B concentrate.

(b) **ESTIMATION BY GROWTH OF ORGANISMS**—(1) **Yeast.**—It was suggested by Funk and Dubin (1920) that it might be possible to estimate quantitatively the presence of vitamin B₁ by means of the growth of yeast, since vitamin B₁ was known to provide a growth stimulus for yeast. About the same time, Williams (1920) published a similar method. He, however, as well as Funk and Dubin, noted the adverse effect of inhibitory substances.

(2) **Streptothrix Corallinus.**—A test which is applicable to the testing of antineuritic concentrates after the charcoal adsorption stage has been evolved by Orr-Ewing and Reader (1928). Reader found that *Streptothrix corallinus* would not grow in carefully purified glucose. A specimen of Peters' torulin, however, proved a powerful growth-promoting agent. By comparing the growth of the streptothrix in test flasks, containing various solutions of standard torulin, it was found possible to test for the presence of the vitamin in solutions too dilute for pigeon tests.

Later experiments by Kinnersley, Peters, and Reader (1928) have shown that the curative factor for pigeons is not identical in every respect with the factor which promotes the growth of *Streptothrix corallinus*.

XV. QUANTITATIVE ESTIMATION OF VITAMIN B₂.

Since the separation of the vitamin B complex into several factors it has been found necessary to devise a method of testing the antineuritic factor separately.

Chick and Roscoe (1928, 1929) have supplied vitamin B₂ in the form of either autoclaved yeast (0.49 gram daily) or coagulated egg-white in quantities sufficient to supply the protein content of the diet. Growth ceases after 2 to 3 weeks, but is resumed if a source of vitamin

B₂ is fed. The minimum dose of B₂, which gives an average weekly increase of 10 to 12 grms. in weight, is suggested by these workers as a standard for comparison.

The source of B₁ used is Kinnersley and Peters' "torulin" yeast fraction, which is practically devoid of vitamin B₂. Eddy (1928) describes a method of vitamin B₂ estimation, using a preparation obtained by Williams by refinements in the control of the adsorbing power of fuller's earth as the source of vitamin B₁. With rats the preparation produces, when used as the sole source of vitamin B in an otherwise adequate diet, a slight amount of growth. Autoclaved yeast alone also stimulates growth at first, but a growth that is soon arrested and followed by death from polyneuritis. By combining Williams' preparation with an adequate supply of autoclaved yeast, normal rat growth is obtained. As an example of his method he describes the differentiation of vitamin B₁ and vitamin B₂ in the banana.

By the Sherman test, Eddy (1927) showed that 8 to 10 mgrms. of ripe banana per day was necessary to prevent polyneuritis and to secure a growth of 20 mgrms. gain in 60 days. Using Williams' preparation, which is a source of vitamin B₁ free from vitamin B₂, 2 grms. of banana per day supplies enough vitamin B₂ to produce even more than 20 grms. gain in 60 days, while 6 grms. supplies enough for almost normal growth. The banana is, therefore, richer in vitamin B₂ than B₁.

An attempt has been made by Munsell (1930) to obtain a basal diet which would not involve elaborate methods of preparation and yet be suitable for estimating vitamin B₂. It was found that by using 30 per cent. of white corn in the Sherman and Spohn vitamin B-free diet, an adequate amount of vitamin B was supplied without adding an appreciable amount of vitamin G.

XVI. QUANTITATIVE ESTIMATION OF VITAMIN C.

The very ease with which vitamin C deficiency can be demonstrated in guinea-pigs constitutes a weakness in its standardisation.

Wieland (1927), working on the effect of ultra-violet irradiation on the antiscorbutic properties of milk, points out that caution must be exercised in applying to man the results of experiments with guinea-pigs, owing to the extremely high susceptibility of the latter to vitamin C deficiency.

In 1907 Holst carried out experiments to determine the antiscorbutic value of foodstuffs. He took as the unit, or minimum protective dose, the amount that would protect a guinea-pig weighing from 150 to 300 grms. from manifest signs of scurvy.

Since Zilva (1925-1928) has succeeded in demonstrating that the vitamin activity of lemon juice is associated with a solid residue, which represents about 0.5 per cent. of the fresh lemon juice, it has been possible to estimate more definitely the amount of vitamin C necessary to protect from scurvy. The methods chiefly used are:

(1) **SHERMAN'S METHOD** (1922).—Sherman's unit quantity of antiscorbutic was defined as the smallest quantity of food source necessary to total absence of scurvy symptoms, limiting the time of the test to 70 to 90 days, using guinea-pigs and the following basal diet:

Ground whole oats	59 per cent.
Baked skim milk powder	30 "
Butter fat	10 "
Salt (NaCl)	1 "

Eddy and Kohman (1924, 1925, 1926, 1928) have used this method in studying a wide range of canned vegetables and fruits, using the following modification of the diet:

Baked skim milk	30 per cent.
Butter fat	9 "
Salt...	1 "
Rolled oats and wheat bran ($\frac{1}{2}$ and $\frac{1}{2}$)	59 "
Cod-liver oil	1 "

plus a certain amount of yeast fed separately.

Another modification of the basal diet, the substitution of egg-yolk for milk, is stated by Bezssonoff (1926) to permit a more rapid and accurate assay of vitamin C content. Still another modification is that of Randoin and Lecoq (1929). They suggest the following diet, which they claim to be simple to prepare and free from complex foodstuffs :

Meat peptone	17	per cent.
Brewers' yeast	3	"
Butter fat	5	"
Maize starch	64	"
Osborn and Mendel salt mixture	2	"
Sodium chloride	1.5	"
Calcium lactate	5	"
Filter paper	2.5	"

The peptone, starch, salts, and filter paper are gently boiled in 130 to 135 grms. water with stirring until about 30 to 35 grms. of water have evaporated. After partial cooling, the yeast and butter fat are added. The final preparation, containing about 50 per cent. of water, is complete except as regards vitamin C, and produces good growth in guinea-pigs for 14 to 18 days, after which the typical symptoms of scurvy develop, death taking place between the twenty-fifth and thirty-second day. The animals eat from 80 to 130 grms. or even 150 grms. per day. The daily addition of 2 c.c. of lemon juice (for guinea-pigs of less than 400 grms.) or 3 c.c. (for guinea-pigs above 400 grms.) renders the diet complete in vitamin C.

Sherman also introduces the rating of scorbutic signs in animals which were not fully protected, and thus he estimated the dose of antiscorbutic given in quarters of the fully protective dose. Even with these improvements, however, his method has been criticised, particularly by Höjer (1926), who has formulated a method of his own.

(2) **HÖJER'S METHOD** (1926).—In 1924 Höjer, extending the observations of Zilva (1919), and the earlier ones of Jackson and Moore (1916), made a study of the antiscorbutic changes in teeth. In 1916 and 1928 he supplemented these studies by a scheme for utilising tooth pathology as a quantitative measure of antiscorbutic value. The method depends upon specific changes in the teeth appearing in 3 weeks or less. The following is Höjer's description of the technique :

"Young guinea-pigs from a certain day on are given a basal diet free from antiscorbutic, but otherwise complete, and to this diet are added quantitatively daily doses of the juice to be examined. For a sharp determination of the fully protective dose it is advisable to have several animals on different doses. In addition there ought to be 2 control animals on the basal diet alone, and 2 on the basal diet with a fully protective dose of a known antiscorbutic. After a period of 10 to 14 days all the animals are killed. The one-half of the lower jaw is taken out and decalcified in a 5 per cent. tri-chloroacetic acid solution. It is then embedded and sectioned after 1 week. The section is made through the foremost molar at right angles to the longitudinal axis of the jaw. Some sections are stained with hæmatoxylin-eosin, and others with tri-oxyhæmatin, according to the method of Hansen."

Such sections, according to Höjer, not only reveal histological pictures characteristic of scurvy or its absence, but it is possible from pictures of partially protected animals to predict the fully protective dose, as shown in table on page 56.

Höjer's method has been compared with Sherman's by Goettsch and Key (1928). They studied particularly the question of whether the tooth changes noted had any correlation with symptoms of rickets, and found none. The actual tooth changes were as described by Höjer. Höjer himself claims the following advantages for his method :

(1) Accuracy in fixing the fully protective dose.

(2) Shorter time required—3 weeks instead of 3 months—and, therefore, economy.

The studies of Goettsch and Key (1928), also of Eddy and co-workers (1929), show that if the tooth is a true index of antiscorbutics, it is a much more sensitive one than the Sherman

TABLE 1.—DETERMINATION OF THE ANTISCORBUTIC VALUE OF FOOD BY HÖJER'S METHOD.

										Part of Protective Dose.
1. Dentine of normal size ; its inner and outer layer uniformly coloured ; predentine regular, uncalcified ; dentine and predentine holding collagen :										
(a)	Odontoblasts long, slender, parallel, of equal height	1.0
(b)	Odontoblasts partly shorter...	0.9
2. Dentine very thin, uniformly coloured ; odontoblasts parallel, short										
(The degree of osteoporosis, hyperæmia, and collagen permits the distinguishing of different stages, but not very sharply. Those animals whose dentine formation seems to have been arrested are therefore excluded)										
3. Dentine in inner and outer layer differently coloured :										
(a)	Odontoblasts on the larger pole of the incisor cross-section short and parallel	0.8-0.3
(1)	The Tomes' canals going parallel through a normal predentine	0.8
(2)	The uncalcified predentine defective formation of network bone beginning	0.7
(3)	Uncalcified predentine lacking	0.7-0.5
(4)	Network bone formation greater	0.4
(5)	Tomes' canals in the old dentine widened	0.3
(b)	Odontoblasts on the larger pole of the incisor cross-section no longer in continuous layer	(0.3-) 0.2-0.0
(1)	Tomes' canals in the outer layer of new bone	0.2
(2)	No Tomes' canals in the new bone, osteoporosis and hyperæmia well developed	0.1
(3)	The old odontoblasts in greatest disorder, osteoporosis, and hyperæmia very evident	0.0

A tooth section showing the picture 3 (a) (1), with 0.5 gram of antiscorbutic daily would permit prediction that the fully protective dose of that antiscorbutic would be 0.622 gram (0.5 gram = 0.8x).

scoring system, for whereas the Sherman method fixed 1.5 c.c. as the protective dose for orange juice, prevention of the scorbutic symptoms in teeth required a daily dosage of 3 c.c. Similarly, in testing banana, Eddy and co-workers found the antiscorbutic dose for tooth protection double that previously obtained as minimum protective dose by the Sherman method—10 grms. instead of 5 grms.

XVII. QUANTITATIVE ESTIMATION OF VITAMIN D.

(a) **BIOLOGICAL.**—For experiments on vitamin D, Lesné and Clément (1928) strongly recommend Pappenheimer's Diet 85, deficient in phosphorus, and consisting of :

Starch	80.9
Egg albumen	10
Butter	5
Salt mixture	4.1

The animals must be kept in the dark throughout the experiment.

(1) **The Line Test.**—The chief biological assay of vitamin D is based upon the "line test," described by McCollum, Simmonds, Shipley, and Park (1922), and elaborated by Coward (1928). This test depends upon the appearance of a "line" or shadow of calcification, which shows a light strip on an X-ray photograph of a rachitic joint when antirachitic agents are given.

Basing their method on this test, the Pharmaceutical Society of Great Britain has adopted a particular sample of irradiated ergosterol as a standard, and has suggested that biological assays of the vitamin D content of other preparations might be made by referring their potency to that of this standard. The vitamin D unit would be defined as the amount of activity possessed by a certain quantity of this standard preparation, and the potency of other preparations in terms of units might then be expected to have some general value. The strength of this standard sample of irradiated ergosterol is such that a daily dose of not more than 0.0001 mgrm. will cause complete healing of the rickets induced in rats under the conditions described.

Possible modifications of the technique of Coward have been suggested by Schultz (1929).

His chief criticisms of the Coward technique are that under the conditions of her test there is no real evidence that the experimental animals have developed rickets to the same extent, and that only 1 rat is employed for each of the four or five graded amounts of cod-liver oil or other preparation under examination for vitamin activity. He therefore suggests an alternative technique which he has employed with success over a long period for the assay of different preparations of the vitamin. The chief features of this technique are the employment of young rats from the same breeding stock and of the same age (27 days). The animals are maintained on the McCollum 3143 diet * for 14 days, and only those with X-ray-established rickets of similar severity selected for the test. Graded quantities of the substance under examination for vitamin activity are then added to the diet, 4 animals being used for each dosage. After 21 days (from the commencement of the experiment) the animals are again X-rayed. The quantitative value of each dosage is estimated by examination of the corresponding X-ray photographs before and after administration of the test substance.

In order to obtain uniform results when the animals lose weight, which is always emphasised as tending to prevent the development of rickets, Coward points out the great importance of having a post-lactation, pre-experimental period as carefully controlled as the experimental period.

(2) **Calcification of the Knee-joint.**—In the method of estimating vitamin D, recommended by Poulson and Lovenskold (1928), young rats are fed for 25 days on a diet consisting of yellow corn 76 per cent., wheat gluten 20 per cent., calcium carbonate 3 per cent., and sodium chloride 1 per cent. An X-ray photograph of the knee-joint is then taken (under light anæsthesia with ether). The substance to be tested is then added to the diet, another X-ray photograph being taken at the end of the test period. The antirachitic action of the substance is then judged by comparing the pictures taken at the beginning and end of the test period. It is essential that growth be maintained during the test period, and rats which do not show an increase in weight are discarded.

(3) **Calcification of Femur.**—Sherman and Stiebeling (1929) consider that calcification of the femur is a definite criterion in determining proportions of vitamin D in food, and have shown the advantage of such experiments upon rats immediately after separation from their mothers. Young rats were used, separated from their mothers at 21 days and 28 days. At 28 days rats were used in three experimental periods—28th to 56th day, 52nd to 80th day, and 110th to 166th day. The 21-day rats were used in periods, 21st to 56th day, and 52nd to 80th day.

Weekly determinations were made, and at the end of the period femurs were dissected and ash and calcium determined.

All the criterions used in other experiments were employed here—percentage of ash, or of calcium in fresh bone, ash or calcium in the alcohol-ether extracted bone, ratio of ash to organic residue of the bone. In the experiments graded allowances of whole milk powder resulted in an increased deposit of calcium in femurs both from the time of separation to the 50th day of age, and from the 52nd to the 80th day of age. Calcium deposit of the femurs was found to be more in proportion to the supplements of vitamin D than are the weight gains.

(4) **Disappearance of Rachitic Symptoms in Children.**—The different degrees of severity of rickets are manifested by the state of calcification of the knee-joint. Since Rosenheim and Webster (1926) have succeeded in producing vitamin D by the action of ultra-violet light upon ergosterol the standardisation of antirachitic vitamin has become still more accessible. Acti-

* The McCollum 3143 diet consists of :

Whole wheat kernel	33.0
Whole maize kernel	33.0
Gelatin	15.0
Wheat gluten	15.0
NaCl	1.0
CaCO ₃	3.0

vated ergosterol is a hundred thousand times as potent as cod-liver oil and is able to prevent or cure rickets in rats in a daily dose of 1/20,000 mgrm. or less. This potency has been confirmed by Holtz and Gyorgy (1927, 1928) in tests on rats, and by Hottinger on puppies.

Hess and Lewis have, during 1928, been standardising the effects of the antirachitic vitamin on children. The usual daily dose for cure has been equivalent of from 2·5 to 5 mgrms. of irradiated ergosterol. The criteria of effective dosage were calcification of the cranial bones, and of the epiphyses, disappearance of beading of the ribs (though they emphasise the fact that this sign is confusing as a gauge of healing), improvement of appetite, and diminution of excessive sweating.

The question of dosage of irradiated ergosterol is an important one in view of the difficulty of appraising the strength of various commercial preparations of it.

Biologic tests carried out by Hess and co-workers (1928, 1929) tend to show that it is readily absorbed. They extracted with ether the urine and faeces of an infant which had been receiving about 5 mgrms. daily of irradiated ergosterol, and fed the extract to rats which were on a rachitogenic diet. It was found that little or none of the irradiated ergosterol was present in the excreta.

They formulate a tentative statement as to the correct dosage of irradiated ergosterol as follows: For a baby growing at the normal rate, a daily dose of about 0·5 mgrm. as a prophylactic and 1 mgrm. for the average case of rickets. These amounts are equivalent to about 7 and 14 teaspoonfuls daily of high grade cod-liver oil. They consider it advisable not to prescribe it for the newborn infants, or to do so guardedly, although Vogt recently has reported favourable results in newborn infants that received from 2 to 5 drops of a 1 per cent. solution (about 1 to 25 mgrms.).

The protective or curative dose of the rate of an average preparation of irradiated ergosterol is about 0·0001 mgrm., so that 2000 "rat units" of this sterol is equivalent to about 0·2 mgrm. The tables on page 59 show the results of the experiments of Hess and Lewis by the biological method of testing the potency of irradiated ergosterol.

(5) Protective Method.—A protective rather than a curative method is advocated by Scheunert and Schieblich (1929) in testing substances for vitamin D. The following method is suggested: Young rats (35 grms.) are fed on a rachitogenic basal diet to which varying amounts of the substance to be tested are added. Ten rats are used for each amount, and 10 form the control group, receiving no addition to the basal diet. After 14 days the animals are examined by X-rays. The unit of antirachitic potency is taken as the minimum amount of vitamin D which will, under these conditions, definitely protect at least 8 out of the 10 rats from rickets. Of the control group, at least 9 rats must show definite rickets. Vigantol was found to contain 25,000 units of vitamin D in 1 c.c., radiostol 2000 units per cubic centimetre, and präformin 800 to 1000 units per cubic centimetre.

THE NEW STANDARD FOR VITAMIN D.—The Medical Research Council have recently (1930) issued a new standard for vitamin D. The necessity for such a standard was emphasised as follows: Any unit or standard based directly on the response of the experimental young rats to administration of antirachitic material is unsatisfactory, owing to the disproportionate variation in the response to equal doses of apparently similar animals, when tested at different seasons of the year or bred in different laboratories. The vitamin D unit should rather be referred to a stated quantity of some standard antirachitic material, with which comparison can be made by any reliable biological method.

They have chosen as their standard a preparation made by exposing pure ergosterol to ultra-violet light under carefully determined and measured conditions which can be repeated at will. The estimation of vitamin D in any material is to be performed by comparing its action with that of the standard in similar experimental animals under the same conditions.

TABLE 1.—EFFECT OF IRRADIATED ERGOSTEROL ON INFANTILE RICKETS AND TETANY.

Case.	Age. Mos.	Wt. Lbs.	Antirachitic Treatment.	Date.	Cranio- tabes.	Bead- ing.	Blood.		Röntgenograms.	Comment.
							Cal- cium, mg. per 100 c.c.	Phos- phorus (Inor- ganic) mg. per 100 c.c.		
M. G.	5	12½	Ergosterol, 2.5 mgrms., 3/1-3/23	3/ 1 3/15 3/23	+++ +± ±	± ± ±	7.0 10.9 —	6.7 6.6 —	— Healing rickets Marked healing	Latent tetany
G. Y.	2	6½	Cod-liver oil, 15 grms., 1/16-2/20	12/ 5	0	0	—	—	—	Triplet; latent tetany; failure of cod-liver oil
	4½	9½	Ergosterol, 4.0 mgrms., 2/21-3/14	2/21	++	++	8.3	6.2	Mild rickets; osteo- porosis	
	5½	11		3/14	+	+	11.4	5.4	Healing	
B. Y.	2	5½	Cod-liver oil, 15 grms., 1/16-2/20	12/ 5	0	±	—	—	—	Triplet; failure of cod-liver oil; rickets in spite of nor- mal calcium and phosphorus
	4½	8½	Ergosterol, 2.5 mgrms., 2/20-3/15	2/21	+++	++	10.1	6.3	Very slight rickets; osteoporosis	
	5½	11		3/15	±	++	11.4	9.6	Slight healing	
I. V.	7	11	Ergosterol, 6.0 mgrms., 4/4-4/13	4/ 4 4/13	+++ +±	+± +±	6.4 10.1	4.7 4.7	Marked rickets; osteoporosis	Latent tetany; improved ap- petite; gain in weight; im- provement of musculature
C. H.	9	12½	Vitaglass, 2/13-3/15	1/16	+++	+	10.0	4.4	Definite rickets	Twin; improvement of cranio- tabes with progressive rickets
	11	15	Ergosterol, 2.5 mgrms., 3/16-4/1	3/16 3/29	± ±	++ ++	9.9 11.2	4.2 6.2	Rickets worse Healing	
M. D.	4½	9½	Cod-liver oil, 15 grms., 1/27-2/16	10/30	0	0	—	—	—	
			Ergosterol, 4.0 mgrms., 2/16-3/14	2/16	++	+±	7.8	3.8	Mild rickets; osteoporosis	Twin; latent tetany, failure of cod-liver oil Diminished phosphorus in urine
	5½	10½		3/14	±	+±	11.6	10.4	Healing	
F. H.	2	6	Ergosterol, 0.5 mgrms., 5/4-6/19	5/ 3	++	+	—	—	Mild rickets; osteoporosis	Premature infant; hyper- calcemia with progressive rickets; increase of irradi- ated ergosterol brought about healing within a week
	3	8½	Ergosterol, 2.5 mgrms., 6/19-6/25	6/19	+ + ±	+ ±	13.6	4.2	Rickets worse	
	4½	9½		6/25	+ to + ±	+ ±	12.3	6.0	Healing	

TABLE 2.—EFFECT OF IRRADIATED ERGOSTEROL ON THE CALCIUM AND PHOSPHORUS CONTENT OF THE BLOOD IN NORMAL INFANTS.*

Case.	Sex.†	Age. Months.	Weight. Lbs.	Ergosterol.		Blood.	
				Number of Days Given.	Daily Dose.	Calcium, Mgrm. per 100 c.c.	Phosphorus (Inorganic) Mgrm. per 100 c.c.
L. R. ...	♂	9½	18½	—	—	10.9	5.6
				61	2.5	12.44	4.53
				77	2.5	13.8	6.85
J. A. ...	♂	6½	15½	77	0.5	15.3	6.05
Y. G. ...	♀	9	16	—	—	10.45	4.45
				7	5.0	12.2	4.0
R. S. ...	♂	9	19	—	—	11.0	6.7
				12	5.0	12.62	4.7
				24	5.0	12.3	6.0
M. G. ...	♂	7	15	—	—	12.1	6.2
				11	5.0	13.1	4.06
				23	5.0	14.7	4.4
M. G. ...	♀	6½	11½	59	2.5	14.3	6.8
M. S. ...	♀	9	14	80	2.5	12.7	6.0
M. S. ...	♂	7	17	80	2.5	13.6	5.8
H. S. ...	♂	2	11	73	0.5	13.5	6.0
M. G. ...	♂	7	15	80	0.5	11.9	5.8
S. N. ...	♀	7	19	80	0.5	11.9	5.34
M. R. ...	♂	8	18	80	0.5	14.2	5.52

* These estimations were made in the spring and early summer. The percentages may have been heightened by the effect of solar irradiation.

† In this column, ♂ indicates male; ♀ female.

By this means the variable factor, the experimental animal and its environment, should be obviated as much as possible. The following resolutions have been adopted by the Committee :

(a) That the standard solution of irradiated ergosterol prepared and maintained by the National Institute for Medical Research be recommended for adoption as a standard for the comparative estimation of vitamin D.

(b) That the unit of vitamin D be defined as the antirachitic potency of a quantity of this preparation corresponding to 0.0001 mgrm. of the ergosterol used in its production.

(c) That either the X-ray method, the "Line test," or chemical analysis of the bones of the experimental animals, be recommended for use in the estimation of vitamin D.

(d) That supplies of the standard material be made available for general distribution from the National Institute for Medical Research, Hampstead. As the supply is limited it is hoped that users will themselves prepare standard solutions of irradiated ergosterol for use in individual tests, retaining the National Institute's material for reference. It is important that all such standard materials be kept at temperatures not exceeding 0° C.

The new unit has the same value, in terms of the weight of the original ergosterol represented, as that used for some years past in the Pharmaceutical Society's Laboratories, on the basis of the provisional standard solution supplied to them. This provisional standard may differ slightly from the new one now to be supplied, but the new unit will, in spite of the possible difference thus introduced, correspond to one of those already current.

(b) **CHEMICAL METHODS**—(1) **Blood Calcium and Phosphorus**.—Hess and Lewis (1928) have taken advantage of the second method of estimating vitamin D—the chemical method, as evidenced by the percentage of calcium and inorganic phosphorus in the blood. In this connection they have made the extremely important observation that there is a marked distinction between the action of the physiologic and the toxic dose of irradiated ergosterol. In several infants, receiving 5 mgrms. daily, the calcium rose abnormally high; in one case, after less than 1 mgrm. daily for about 6 weeks, to a level of 13.6 mgrms. They conclude that in some cases of rickets and mild tetany irradiated ergosterol, as prescribed at the present time, may bring about either an excessive increase of calcium or of inorganic phosphorus, or of both—a hyper-mineralisation.

(2) **pH of Faeces**.—Another chemical test for vitamin D was suggested by Jephcott and Bacharach in 1926, but it does not seem to have been widely adopted. They found that the feeding of cod-liver oil or irradiated cholesterol to rats, or the irradiation of the rats themselves, causes alkaline faeces to become acid again, and they suggested that the change in reaction might be used as a test for antirachitic substances if no alteration were made in the diet, which in itself would change the reaction of the faeces.

More recently (1928, 1929) these workers have met the objection of certain observers that the test may not be quantitative or even specific, with the statement that the conditions originally laid down for the test have not been strictly carried out. They maintain that the test, if made with concentrated preparations of the vitamin on healthy animals with diets, length of test, and method of pH determination as originally described, may be applied to the quantitative determination of synthetic vitamin D as well as of the naturally occurring factor.

(3) **Colour Reactions**.—Several colour reactions for the presence and quantity of vitamin D have been suggested, using as a standard cod-liver oil which had been oxidised until the tests with AsCl_3 and P_2O_5 , proposed by Rosenheim and Drummond, for vitamin A were negative. These tests will be described in detail under the heading of vitamin D.

(c) **OPTICAL METHODS**.—A spectrographic method for determining the activity of anti-rachitic preparations has been described by Tixier (1928). When solutions of ergosterol are exposed to ultra-violet irradiation certain wave-lengths produce an increased transparency

of the solutions. On prolonging the irradiation, the transparency increases even after the antirachitic activity has attained a maximum or commenced to diminish.

In Tixier's method a solution of irradiated ergosterol is placed in a quartz vessel designed so that the thickness of solution in front of the slit of the spectrograph can be varied and accurately measured. The thickness of solution of ergosterol necessary to extinguish the ray of wave-length $253.7\mu\mu$ given by a mercury-vapour lamp producing radiations of constant intensity is determined. With a 1 per cent. solution of ergosterol, Tixier obtained the following results: Before irradiation the extinction thickness was 0.16 mm., at optimum activation (determined by biological tests) the thickness was 0.20 mm., and at maximum transparency the thickness was 0.23 mm.

Fabre and Simonnet (1929) have recently found, however, that various preparations of vitamin D obtained by irradiation of ergosterol, under different conditions, and shown by biological tests to be equal in antirachitic potency, gave different types of absorption spectra. The absorption is modified by the impurities accompanying the vitamin D. They suggest that it is premature to use the optical method as a test of the antirachitic power of preparations of the vitamin.

XVIII. QUANTITATIVE ESTIMATION OF VITAMIN E.

Vitamin E can be quantitatively estimated from two points of view: the cure of sterility which has been brought about in either male or female, or both animals; and the prevention of foetal resorption when pregnancy has taken place.

Pryde (1928) states that in rats suffering from sterility, due to lack of vitamin E, a curative daily dose of wheat germ oil (the richest source known) is 25 mgrms., but a single massive dose of 500 mgrms., representing 22 daily doses, is also curative, even when given on the fifth or sixth day of pregnancy, which would otherwise fail through foetal resorption.

XIX. CLASSIFICATION OF VITAMINS.

It appears from the result of recent researches that progression in the knowledge of vitamins will ultimately be made from the chemical standpoint, and the most general and non-committal method of classification would therefore seem to be according to their solubility, *i.e.* fat-soluble and water-soluble. According to this classification, they are then still confined within the two groups in which they were placed during the first period of investigation, ending with the division of the known vitamins into the three varieties—fat-soluble A and water-soluble B and C, in 1924. At the present time the fat-soluble group includes vitamins A, D, and E, while the water-soluble varieties are the vitamin B complex and vitamin C.

In addition to these, the French workers recognise a vitamin which Wildiers (1901) has called "bios," in its connection with yeast, though a class of similar substances is supposed to be distributed among vegetable extracts. "Bios" is characterised by its property of stimulating the growth of yeast cells in artificial culture media. Thus it is claimed that the growth of yeast cells is catalysed by the presence of "bios" in sufficient amount: if the number of cells in the yeast inoculum is not sufficient to make up the necessary supply of "bios," growth cannot proceed normally. Drummond, however, draws a distinction between substances which accelerate or stimulate growth and substances which are essential for growth. According to his definition of a vitamin, then, "a substance which is essential to the life and well-being of an organism which does not possess the power to synthesise that substance, and also is organic in nature, and does not belong to any one of three great classes of foodstuffs—proteins, fats, and carbohydrates"—"bios" is not a vitamin. The vitality of "bios" and its resistance to the time factor is shown by the observations of Yoshitoshi (1928), who has found a consider-

able amount of "bios" in lotus seeds which have been buried for several hundreds of years in a peat bed near Pulantien, Manchuria, and which still possess germinative possibility.

According to recent researches of Narayanan (1930), he has isolated a "bios" concentrate from yeast extracts which produce marked stimulation of yeast growth in doses of the order of 0.01 mgrm. per cubic centimetre of an artificial sugar salt medium. Since neutral lead acetate was found to precipitate vitamin B₂ from baryta hydrolysates of yeast extracts, leaving "bios" in solution, "bios" cannot be considered identical with vitamin B₂.

The existence of a new vitamin, called "Z," by Eckstein and von Szily (1925), has not been confirmed. They obtained a cataract in young rats on substituting gelatin for wheat gluten in McCollum's rachitic diet, and therefore concluded that the new cataract-preventing vitamin was present in wheat gluten. Stepp and Friedenwald (1924) had, however, not been able to produce the lesion, and Goldschmidt (1927) showed that Eckstein and von Szily had mistaken for a cataract the simple corneal modification which occurs when the lens of young rats is exposed to the air during the first 10 days after birth. Yet another vitamin, so far unnamed, has been foreshadowed by Wesson (1927), who states that lard contains a vitamin which permits the assimilation of carbohydrates. When this supposed substance is absent there occurs abnormal transformation of carbohydrates into fats, even when the diet consists only of water and dextrin.

PART II

THE FAT-SOLUBLE VITAMINS

PART II

THE FAT-SOLUBLE VITAMINS (VITAMINS A, D, AND E)

VITAMIN A

The first knowledge of the fat-soluble vitamin A was based on qualitative experiments which showed that its distribution in nature occurred chiefly in animal fats, such as butter, egg-yolk, ox-fat, and particularly in cod-liver oil.

The difficulties of its investigation have been considerably increased by the fact that during the early years the presence of vitamin D was not recognised as a separate factor.

Much of the work on vitamin A, for example, that of K. H. Coward and Drummond (1921), on the formation of vitamin A in living plant tissues under the influence of light, with no necessity for oxygen or chlorophyll, has had to be re-examined since the discovery of vitamin D.

XX. OCCURRENCE OF VITAMIN A.

The ultimate source of vitamin A is the green plant, and the most recent work indicates that it may be formed in the complete absence of light, but it is certain that light can accelerate its synthesis, for green plant tissues are better sources than white.

The content of vitamin A of animal tissues depends on three factors: (1) The amount of vitamin A in the food ingested by the animal, since animal tissues cannot synthesise vitamin A. (2) The amount of adipose tissue in the animal from which the fat is derived. (3) The nature of the tissue, with regard to its function of reserve, transformation, or elimination. Thus, liver is rich in vitamin A by virtue of its special function in utilising lipoids, kidney by virtue of their elimination when the food contains more than is necessary for the needs of the organism.

Vogt (1929) has drawn attention to the presence in large amounts of vitamin A in the foetal liver. He states that the vitamin A appears to occur solely in the liver in the foetus, and that the liver must play an important part in its metabolism.

In plants, where it is synthesised, it seems to be less distinctly associated with the fat, and Pryde (1928) suggests that this may be due to the fact that in the plant it is associated with other molecules which are not fat-soluble. A peculiarity of the vitamin A exchange in the sea is that the main source of vitamins for deep-sea animals is the unicellular algæ, and that the concentrations of vitamin A attained are much higher than occur on land. The following table shows the source of vitamin A and the approximate weight of substance in grams required to produce growth in rats weighing 100 grms:

Source.	Approximate Weight.
<i>Marine sources:</i>	Gram.
Diatom (<i>Nitzschia closterium</i>) (dry weight) ...	0.04
Plankton (dry weight)	0.1
Small fish (capelin, <i>Mallotus villosus</i>) ...	0.1
Cod-liver oil	0.003-0.01
Cod's roe (male)	0.025
<i>Land sources:</i>	
Green vegetables (cabbage) (dry weight) ...	0.1
Butter	0.1-0.2
Ox liver	0.25

(a) **VITAMIN A AND CHLOROPHYLL.**—The relation between the vitamin A content of green tissues and the intensity of its chlorophyll function have been carefully investigated by several workers. The data from these experiments support the conclusion that the vitamin A content of plant tissue is associated with its greenness.

Dye, Medlock, and Crist (1927), using leaf and head-lettuce as the source of vitamin, found the outside green leaves superior in growth-promoting properties to the inside yellow leaves.

Later experiments by Crist and Dye (1929) on green and bleached asparagus support the observations on lettuce. Green asparagus, whether fresh, freshly cooked, or canned, when fed daily at the rate of 0.1 gram per animal, contained vitamin A in quantity sufficient to promote health and growth in albino rats. Fresh bleached asparagus, when fed daily at the rate of either 0.1 or 0.5 gram per animal, gave no stimulus to health and growth. The animals died as rapidly as the negative controls. Cooking in open kettle fashion effected an improvement in the nutritive quality of bleached asparagus, though not rendering its value comparable to that of the green product cooked in the same manner.

The relation of the vitamin A content to the greenness of alfalfa has been observed by Steenbock, Hart, and co-workers (1925), and later (1929) by Russell. Russell's experiments were undertaken to determine the difference in vitamin A content according to the method of curing the alfalfa. It was found that the vitamin A potency of machine-dried alfalfa, which preserved its green colour, was seven times that of a field sample, dried by exposure to sun and air with consequent loss of greenness.

Recent work by Collison and co-workers (1929) on the vitamin A content of cabbage leaves shows that green cabbage yields a more active product than white. Since extraction of the unsaponifiable residue with alcohol, which removes some vitamin A, still leaves behind a fraction which is potent, these workers consider that evidence is accumulating to show that there may be more than one substance in green leaves possessing vitamin A activity.

Kashan, Krasnow, and Harrow (1928) also conclude that vitamin A is synthesised during the greening of corn, since rats fed on germinated or ungerminated corn lost weight and developed xerophthalmia, while those on the green seedlings gained in weight and were free from xerophthalmia.

Similarly in Widmark's (1924) experiments on two varieties of barley, the one which contained no chlorophyll was found deficient in vitamin A, while that which was rich in chlorophyll was more active.

(b) **EFFECT OF LIGHT ON SYNTHESIS OF VITAMIN A.**—Drummond and Coward (1921) concluded from their experiments on the chlorophyll function of plants that green algæ cultivated in an aseptic mineral medium were capable of synthesising vitamin A, and that etiolated shoots were partially or entirely inactive, while non-etiolated they possessed a greater activity, weight for weight, than that of the grains from which they were derived.

Coward (1927) also states that shoots grown in the dark from wheat seedlings contain more vitamin A when grown slowly at a low temperature than when grown more rapidly at a higher temperature. Moore (1928), however, has shown that etiolated shoots contain vitamin A and that, therefore, light is not essential for the formation of vitamin A in plant tissues. Fresh, etiolated wheat shoots, 10 days old, fed to rats on a diet deficient in vitamin A but adequate in vitamin D, at the rate of 30 shoots per day (equivalent to 1.5 gram of dry wheat) prevented death and restored growth. Seeds fed at the same rate were ineffective. The vitamin D of the diet was supplied by daily doses of 5 mgrms. of irradiated cholesterol. The result is in agreement with statements by Wilson (1922) and Coward (1927) that vitamin A may be formed in etiolated tissues. Etiolated plant tissues are, however, very sensitive to diffuse light, so that in Moore's later experiments the feeding was carried out under a minimum

of red-light illumination. Resumption of growth was obtained in rats on an A-deficient diet by feeding 15 shoots daily—a finding which confirms the view that these shoots are definite but unsatisfactory sources of vitamin A. Even when large quantities of the shoots are fed, the growth and general condition seems to be no better than with small doses. An exposure of some shoots for some hours to the source of red light used in the experiment resulted in quite definite greening. If chlorophyll can be synthesised by this means there still remains a faint possibility that vitamin A may be synthesised during even shorter exposures.

Bezssonoff (1927) states that vitamin A is more abundant as the tissues are richer in chlorophyll, though it is actually neither chlorophyll nor carotin. It does not appear when plants are transferred from the light to darkness. Vitamin A readily absorbs oxygen, and can be extracted from green tissues along with chlorophyll by treating the sap obtained from green tissues with a solution of lead acetate. The precipitate when filtered and dried and treated with ether for 24 hours yields chlorophyll, while vitamin A is found in solution in the ether.

The relative synthesis of vitamin A in sunlight and darkness, and the effect of light from ultra-violet, mazda, and arc lamps have been studied by Heller (1928). Rats and guinea-pigs were used as test animals.

Seeds grown in sunlight contained larger amounts of vitamin A than did etiolated seeds, the amounts seemingly being dependent on the degree of illumination and the proportion of short waves present. The vitamin likewise developed in artificial light but in a smaller amount, the quantity probably being in direct proportion to the intensity and duration of illumination.

According to Coward (1927) and Bezssonoff (1927) the formation of vitamin A in living plant tissues is accelerated by radiating with quartz mercury-vapour lamp.

(c) **VITAMIN A AND XANTHOPHYLL.**—That vitamin A is not identical with xanthophyll is evident from the results obtained by Willimott and Moore (1927). Crystalline xanthophyll, prepared from nettle leaves and fed to rats at a level of 3 mgrms. daily, was ineffective as a source of vitamin A.

Willimott and Moore (1927) have also come to the conclusion, having regard to the variable A-vitamin content of certain grains and roots (even though all grains and roots are poor in comparison with the leaves), that light is necessary for the formation of vitamin A in plant tissues, but not oxygen, carbon dioxide, nor chlorophyll.

(d) **VITAMIN A AND CAROTIN**—(1) **Relationship.**—The relationship between carotin and vitamin A has been the source of many investigations, and it now appears to be more or less universally recognised that experimentally produced avitaminosis A can be cured by the administration of small doses of carotin. Whether carotin and vitamin A are one and the same substance, as Euler and co-workers have been led to suppose, remains at present uncertain. Carotin is widely diffused throughout the vegetable kingdom and has recently been recognised as occurring in milk, butter, eggs, corpus luteum, fat, and blood serum. The higher animals cannot produce carotin synthetically, but obtain it ready-made from vegetables. Carotin is an unsaturated hydrocarbon ($C_{40}H_{56}$) with a strong affinity for oxygen, being transformed into colourless xanthophyll. In pure form it appears in small variable crystals, slightly soluble in alcohol, but most soluble in chloroform, benzol, and carbon disulphide; it gives two characteristic absorption bands in the spectrum, but is inactive to polarised light.

(2) **Vitamin Activity of Carotin.**—That the vitamin activity of carotin is intensely stable is indicated by the experiments of Javillier and Emérique (1930) on a sample prepared from spinach leaves 40 years ago. The specimen had been kept in a sealed tube in an atmosphere of hydrogen, and had been exposed for a long time to a weak, diffused light. It was found that the carotin in daily doses of the order of 1/100 mgrm. was capable of preventing

loss of weight and of curing xerophthalmia and other infections of rats previously maintained on a vitamin A-free diet.

Drummond and Coward (1920) and Stephenson (1920) showed that crystalline carotin, carefully purified, was not an adequate source of vitamin A, and later experiments by Dulière, Morton, and Drummond (1929) still failed to find a physiological action of carotin similar to that of vitamin A.

It is pointed out by Euler and co-workers that in Drummond's earlier experiments the separate existences of vitamin A and vitamin D was not realised, while Moore (1929) states that by using fat containing basal diet, instead of the fat-free diet used by Dulière, Morton, and Drummond, he has found carotin intensely active.

It has been suggested that the discrepancy between Drummond's results and those of other workers may arise from the fact that his carotin, which has a higher melting-point, is the pure substance, while that of Euler, Moore, and others contains a small amount of impurity.

Euler and co-workers (1928, 1929), however, and also Moore (1929), and Collison and co-workers (1929), claim to have used the purest form of carotin obtainable, and still to have found it potent. Euler, etc., conducting their investigation with the most improved technique obtained growth with as little as 0.005 mgrm. of crystallised carotin in animals which had been depleted of vitamin A.

Moore (1929) also found that four different samples of carotin, which had been recrystallised a large number of times, all sufficed to cure xerophthalmia and to restore growth when administered (dissolved in oil) to vitamin A-deficient rats in daily doses of 0.01 mgrm. The activity of carrot fat after removal of much of the carotin was found to be inferior to that of the isolated pigment.

Euler and co-workers stated that the carotin which they found active gave the blue colour with antimony trichloride heretofore alleged to be specific for vitamin A.

Moore has followed up these results by a spectroscopic investigation of carotin and the carotinoids.

Comparing these substances with the "biosterin" isolated by Takahashi (1923, 1925), he summarises their differences and similarities as follows :

	Minimal Physiological Dose (Rat).	Blue Coloration with SbCl ₃ .	Selective Adsorption—	
			At 320 $\mu\mu$ in U.-V.	In Visible.
" Biosterin " ...	0.005 mgrm. (Takahashi)	Positive	Present	Absent
Carotin ...	0.005 mgrm. (Euler, Euler, Hellstrom)	Positive	Absent	Present

In their more recent work on carotin (1930), Euler and Euler state that all the carotinoids which they examined gave the blue colour with antimony trichloride, but that only one (diodo-carotin) has growth-promoting properties. They suggest that the vitamin A of cod-liver oil is a carotinoid or carotinoid derivative, giving a blue reaction of much greater intensity than carotin, and that the vitamin A of vegetables differ from the vitamin A of animal oils. This supposition receives some support from the experiments of Cady and Luck (1930) on the chemical nature of vitamin A. On treating foodstuffs rich in vitamin A with destructive agents, such as sulphur dioxide, distinct differences in reactivity were encountered. The

active principle in cod-liver oil was rapidly destroyed, that of butter was lessened, while that of alfalfa was not appreciably affected. Cady and Luck have concluded that either the active principle in cod-liver oil is not identical with that of alfalfa, or that alfalfa contains substances, absent from cod-liver oil, which exercise a protective function against the destructive action of sulphur dioxide.

Moore suggests that two explanations may be advanced to reconcile the common activity of sources so different in their physical properties: (1) Both carotin and the classical "vitamin A" may independently possess the same physiological action, or (2) physiological action may be due to an active impurity present in each in amounts so minute as not to affect the physical properties of the materials in bulk.

In an attempt to reconcile the divergent results summarised above, Hume and Smedley Maclean (1929) carried out an investigation in order to compare the effect of administering purified carotin, as the sole source of vitamin A, to groups of rats on diets devoid of and containing fat. The use of a natural oil as a solvent for the administration of vitamin A in biological tests has been strongly condemned by Drummond (1929), who recommends ethyl oleate.

Hume and Smedley Maclean therefore considered it advisable to make use of ethyl oleate as a solvent and to examine the behaviour of carotin in it. The carotin was dissolved in ethyl oleate, and the solution was kept in a corked bottle in the cold room when not in use. It was found that in these conditions the carotin was comparatively rapidly decolorised. Thirteen days after the solution had been made up the loss of colour was quite convincing, and no positive biological result was obtained with carotin dissolved in ethyl oleate.

The conclusions drawn from these experiments were as follows:

"The presence or absence of fat from the basal diet does not appear to be a factor of any importance in determining the biological activity of carotin.

"The unsuitability of ethyl oleate as a solvent for carotin affords a sufficient explanation of the discrepancy between Prof. Drummond's results and those of other workers on the activity of carotin as a source of vitamin A. Whether this fact supplies the whole explanation it is not yet possible to say. So far as our experience goes there is at present no evidence available finally to decide the relationship between carotin and the substance responsible for the biological activity in vitamin A of liver extracts. It cannot yet be decided whether carotin acts as a provitamin, as Moore has suggested, or whether carotin from all sources is always accompanied by a constant percentage of impurity, which up to the present has defied all methods to separate it."

It is pointed out by Bezssonoff (1929) that the difficulty of separating vitamin A and carotin is not a proof that they are identical. His own attempts to disprove Euler's hypothesis, by isolating from vegetables an active preparation of vitamin A, free from carotin, have been unsuccessful, but he suggests that explanations such as the selective absorption of vitamin A by carotin, or the existence of molecular combinations of both, are more probable than that of their complete identity.

The chief chemical and physical differences between vitamin A and carotin, which would seem to contradict the supposition that they are identical, may be summarised as follows: (1) Vitamin A must be a colourless compound, since very potent cod-liver oil concentrates are almost colourless, while carotin is highly coloured; (2) vitamin A is freely soluble in alcohol, carotin sparingly so; (3) both compounds give a blue colour with antimony trichloride, but with vitamin A the colour is permanent, with carotin it is fleeting; (4) the absorption spectra are different.

An absorption band at $320\text{ }\mu\mu$, which has been claimed by Morton and Heilbron (1928) to be practically specific for vitamin A, is absent in carotin. Recent experiments by Rosenheim

and Webster (1929), however, seem to show that selective absorption at 320–325 $\mu\mu$, is not a criterion of vitamin A.

(3) **Storage of Carotin.**—Euler, Euler, and Karrer (1929) have stated that carotin administered above a certain level is stored unchanged in the liver of rats. Moore (1930) does not confirm this statement; on the contrary, he finds that carotin, even in doses of the level of 0.75 mgrm. per day, does not appear in the liver in more than minute traces, but the liver of such rats gives an unusually strong reaction for vitamin A, both with the antimony chloride reagent, with the Lovibond's colorimeter, and by spectroscopic examination.

Green and Mellanby (1930) confirm this observation but find the minimal dose required to bring about liver retention considerably higher than that suggested by Moore—at any rate in rapidly growing animals. They suggest that possibly the liver stores its vitamin A as a highly active leuco-form of carotinoid, which may be reconverted to carotin and liberated into the circulation as required.

(4) **The Anti-Infective Action of Carotin.**—Most of the experimental work on carotin and its relation to vitamin A has depended on observations concerning its effect on the weight of rats which have begun to lose weight as the result of vitamin A deprivation.

Green and Mellanby (1930) have carried out experiments to test the action of carotin in its relation to infection in line with their previous observations (1928, 1929) that vitamin A raises the resistance of the body to infection. The results of these experiments, both prophylactic and curative, showed that carotin acts as a very potent anti-infective agent. Complete protection against infection was always found when greater amounts than 0.04 mgrm. of carotin were given daily, even when a prolonged depletion period on a vitamin A-deficient diet had preceded the addition of the carotin. In the case of the animals receiving only 0.01 mgrm. of carotin, the protection against infection was less certain, and some died with organs invaded by micro-organisms. With 0.005 mgrm. of carotin the protection afforded was still less, although even in this case, when the animals were compared with the controls receiving no carotin, there was evidence of an increased resistance.

Green and Mellanby suggest an amount of approximately 0.02 mgrm. as sufficient to restore health and growth. When cabbage was used as a source of carotin, 0.5 gram of the dried leaf, equivalent to about 5 grms. of cabbage, always afforded complete protection. If the generally accepted test of vitamin A be examined, namely, the property of causing growth resumption for a period of 4 weeks after A depletion, it will be seen that the statement of Euler *et al.* (1928) and Moore (1929), that amounts of the order of 0.005 mgrms. of carotin are efficient, may be accepted.

It is apparent from Green and Mellanby's experiments, however, that the degree of protection against infection brought about by this quantity of carotin is only slight, and that ultimately both loss of weight and death with infective foci result. In other words, the protective dose of carotin is higher than that required to stimulate growth for a short period, the minimal amounts for the two purposes being roughly of the order of 0.02 mgrm. (for anti-infective action) and 0.005 mgrm. (for growth resumption).

(e) **VITAMIN A AND LIPOCHROMES.**—The relationship between the content of vitamin A and that of lipochromes in various tissues has been investigated with varying conclusions, the most generally accepted being that the relationship is accidental and not constant.

In the plant kingdom this relationship is more or less constant. The experiments of Steenbock, Boutwell, and Sell (1920, 1921) on maize, peas, carrots, potatoes, and cabbage showed that plant tissues, which carried yellow pigment, were richer in vitamin A than those which contained little pigment, and their observations have been confirmed in other plants by several workers, including Drummond, Coward, Zilva, etc.

In the case of vegetable oils, however, Drummond and Coward (1920) have shown that

the parallelism between pigment and vitamin does not hold good, though their results with palm oil, deeply pigmented, seemed at first to give support to the association theory. On examining other vegetable oils, such as linseed and cotton oils, their results agreed with those of Palmer and Kennedy (1921), who had pointed out that certain vegetable oils, supposed to be rich in lipochromes, were practically destitute of vitamin A. Steenbock and co-workers (1920) had already suggested, as an explanation of some of the exceptions to the theory of vitamin-lipochrome relationship, that the colouring matters might be present in the form of a leuco-compound, and Drummond and co-workers (1920) stated that unless the existence of such a leucoform is assumed it does not appear probable that the fat-soluble vitamin is a member of the lipochrome class of pigments. Their experiments with pale and dark butter and horse fats support this statement, for no definite relationship between the colour and the growth-promoting powers of these fats could be traced. It must be concluded, then, that the frequent association of vitamin A with the lipochrome class of pigments is to be regarded as accidental. Its association with light, however, if the conclusions above referred to are accepted, is interesting in view of the theories of Professor Baly (1925) with regard to photo-chemical reactions. His thesis is that since light of the correct wave-length is capable of converting substances into states of higher energy content, it is in the highest degree probable that sunlight in falling upon the living leaves of plants does more than initiate and carry through the whole gamut of photo-chemical reactions, it maintains the vitality of the plant organism itself. From this point of view vitamin A in green plants may be looked upon, not as a specific chemical substance, which is thereby differentiated from all other compounds associated with it, but as one of these compounds in a state of high activation.

XXI. CHEMICAL NATURE OF VITAMIN A.

(a) **COMPOSITION.**—The end-results of prolonged investigations into the nature of vitamin A go to prove that it is of the sterol class of substances, allied to cholesterol or ergosterol.

Drummond (1919 onwards) has found that the non-saponifiable fraction of the liver fats of mammals, saponified without oxidation, contains all the vitamin A. The vitamin is not precipitable by digitonin, but remains behind in the residue, a thick reddish oil, which contains a mixture of alcohols. Takahashi (1923, 1925) distilled this red oil, and stated that the distillate which he called "biosterin," because it resembled in composition and behaviour the already known cholesterin, was, in effect, vitamin A itself. Further investigation, however, by Drummond and co-workers (1925, 1929) proved it to be only a complex mixture. Drummond carried out the distillate at the low pressure of 0.01 mm. and obtained a series of fractions of highly unsaturated oils of which he considered 95 per cent. were not vitamin A.

The active vitamin is definitely associated with fractions whose end-composition is :

Squalene—a hydrocarbon.

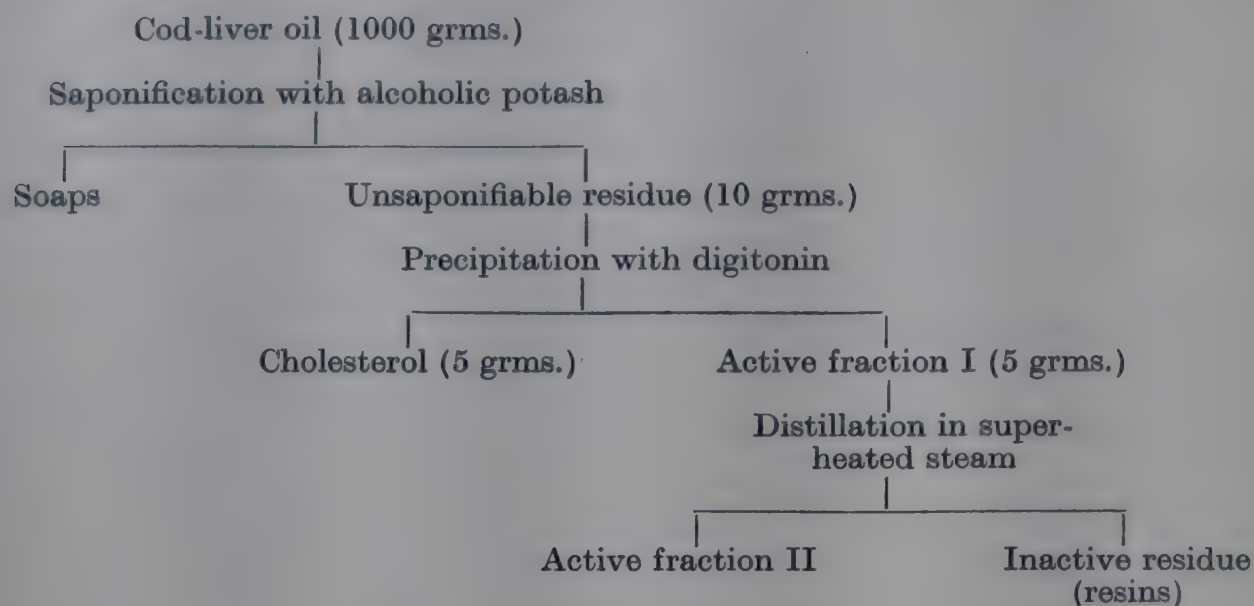
Selachyl alcohol.

Batyl alcohol.

Chimyl alcohol.

None of these constituents have been found to be active in re-establishing growth when fed to rats maintained on a deficient diet, and it must be concluded that the vitamin itself is present in these fractions in a highly concentrated state, just as in the "biosterin" which Takahashi claimed as vitamin A. The actual amount of vitamin found effective is very small, as may be seen from the fact that 20 mgrms. of cod-liver oil in the daily ration is sufficient to ensure growth, this 20 mgrms. representing about 0.2 mgrm. of non-saponifiable matter and about 0.02 mgrm. of the fractions in which the bulk of the vitamin is found after vacuum

distillation. Drummond's method of obtaining the active fractions may be represented as follows :



On re-distilling this " active fraction II " *in vacuo*, 7 fractions are obtained with the following characteristics :

No.	Character.	B.P.	Amount Grms. Per cent.	Iodine Index.	Acetyl Index.	Colour Reaction.
I.	Pale yellow oil	40 (3.4 mm.)	—	—	—	—
II.	Very pale yellow, with an odour of terebene	125–155 (3 mm.)	0.65	114	—	±
III.	Pale yellow oil, with odour of terebene	155–180 (2 mm.)	0.93	120	196	++
IV.	Yellow oil, with strong odour of terebene	184–190 (2 mm.)	0.80	125	120	++++
V.	Yellow oil, with strong odour of terebene	190–220 (1–2 mm.)	4.8	129	174	++++
VI.	Orange-yellow oil, bitter odour	220–270 (1–2 mm.)	3.6	149	140	++
VII.	Reddish brown residue ...	—	—	—	—	—

Another method of the extraction of the vitamin fraction from cod-liver oil is described by Marcus (1928). The method is based on limiting the aqueous content of the soap to the point where the soap is in the form of a magma, and ethylene dichloride is used as the extracting agent.

(b) **SOLUBILITY OF VITAMIN A**—(1) *In Water*.—That vitamin A is not soluble in water has been proved by experiments by Steenbock, Boutwell, and Kent (1918) in contradiction of previous statements by them with McCollum and Simmonds (1917) which they were led to make from observing that butter shaken with water no longer contained vitamin A. The inactivity of the residue, however, was proved to be due to their experimental methods, involving heat and oxidation, which destroyed the vitamin, the watery extract being also inactive. Similarly, watery extracts (both with distilled water and water containing 5 per cent. hydrochloric acid) of whale oil were found inactive by Drummond (1919), and of alfalfa by Steenbock and Boutwell (1920), and in these cases the extracted substances retained their activity.

(2) **In Lipoid Solvents.**—Vitamin A cannot be extracted from vegetable tissues by means of lipoid solvents, such as olive oil, maize oil, or lard, though chlorophyll is so extracted.

(3) **In Ether.**—The solubility of vitamin A in ether depends upon its source, whether animal or vegetable. From animal tissues it is easily extracted, but from vegetable, according to several workers, including McCollum (1917), Osborne and Mendel (1919), Steenbock and Boutwell (1920), it is either not extracted at all, or in very small quantity. Osborne and Mendel (1919), however, state that it is possible to obtain active extracts, corresponding to 1 to 2 grms. of dry leaves, from spinach and lucerne, dried at 60° C.

(4) **In Alcohol.**—According to Drummond (1919), confirming earlier observations of Osborne and Mendel, vitamin A is to some extent soluble in alcohol, and may be removed in small quantity from oils by cold extraction with alcohol.

Steenbock and Boutwell (1920) and other workers have also made alcoholic extracts of vitamin A from carrots, dried alfalfa, and maize; and Simonnet (1925) from cod-liver oil.

(5) **In Acetone.**—Vitamin A is not extracted by acetone (McCollum, Simmonds, and Pitz (1916), and Emmett and Allen).

(6) **In Chloroform and Benzene.**—Both these reagents have been found to extract small quantities of vitamin A (McCollum, Simmonds, and Pitz (1916), Steenbock and Boutwell and Euler (1922)).

(7) **In Mineral Oil.**—That mineral oil acts as a solvent for vitamin A and removes it from the intestine was suggested by the experiments of Dutcher, Ely, and Honeywell (1927), who found that rats receiving a daily allowance of butter fat more than sufficient to supply their vitamin A requirements failed to grow satisfactorily if the butter were mixed with mineral oil before being added to the ration.

Later experiments by Moness and Christiansen (1929), however, are stated by them to give results contrary to those of Dutcher. They found that liquid petrolatum did not interfere with the assimilation of vitamin A.

(c) **COLOUR REACTIONS FOR VITAMIN A.**—Chemical tests have been devised which appear to give an indication of the vitamin A content of an oil.

(1) **Arsenic Trichloride.**—In 1920 it was shown by Drummond that the addition of sulphuric acid to cod-liver oil gave a blue colour, and that this reaction was given not only by cod-liver oil but by the liver fats of all animals. The close relationship between the presence of this reaction and the distribution of vitamin A was also emphasised by Drummond and Watson (1922), and confirmed by Poulsson and Wiedmann (1923), and Sjorslev (1924). This reaction proved too transient to stabilise. It was found also that other reagents, including dimethyl sulphate and trochloracetic acid, which give a red colour when heated with cholesterol, gave a blue colour with cod-liver oil at room temperature. The colour faded in 5 to 10 minutes. Arsenic trichloride, however, was found by Rosenheim and Drummond (1925) to give a more lasting blue colour with oils that contained vitamin A, and the intensity of the colour appeared to be proportional to the quantity of vitamin A present.

The test is performed by adding 1 c.c. of pure arsenic chloride to 1 drop of the oil and shaking the test tube immediately. The oil dissolves to form a clear blue solution; in a few seconds it assumes a purple tint which gradually fades. The reaction is characterised by a well-defined absorption band extending from λ 550 to 590. The sensitiveness of the reaction may be gauged by the fact that it is given by 0.05 mgrm. of oil in the crude state, and by a 1/2,000,000 dilution of the unsaponifiable cholesterol-free fraction. The reasons for believing that the colour reaction is distinctive of vitamin A are that it is given by the same fraction of the oil as is known to contain the vitamin; that it persists in undiminished intensity after distillation with superheated steam in a nitrogen atmosphere; that it is destroyed by oxidation when a current of air is passed through the oil at 100° C.; and that in a series of tests

on over 30 oils and fats a complete agreement was found between the colour intensity and the growth-promoting activity as tested by animal experiment. This colour reaction makes it possible also to distinguish between the growth-promoting vitamin A and the antirachitic vitamin D. From the analogy between the colour reactions of arsenic chloride with cholesterol, and from the possible relation between sterols and lipochromes and their general association with vitamin A in plant tissues, it is suggested that the arsenic chloride reaction is concerned with a substance derived from these types of synthetic plant products under the influence of sunlight.

(2) **Antimony Trichloride.**—Others workers, including Carr and Price (1926) and Willimott and Wokes (1928), consider antimony trichloride in chloroform preferable to arsenic as a reagent (1) because it can be diluted with chloroform, in which the oil itself is soluble; (2) the colour produced is slightly more intense and permanent; (3) it is not so poisonous.

The reaction between antimony trichloride and cod-liver oil consists of a series of colour changes—blue-yellow-red. The blue colour is probably characteristic of vitamin A. The reaction can be retarded by using dehydrated solvents. A rise of 10° C. approximately doubles the rate of reaction.

The following conditions are suggested for the application of this reaction in the quantitative estimation of vitamin A:

“(a) **REAGENT.**—A saturated solution of pure antimony trichloride (recrystallised from chloroform), concentration 26 or 27 per cent. weight in volume in anhydrous chloroform. The reagent should be protected from light and moisture, and decanted for use. Two c.c. to be put in a clean, dry $\frac{1}{2}$ -inch cell.”

Evers (1929) suggests that the solutions of the reagent should not be more than a month old. The quantity of oil taken for the test has a very marked effect on the results obtained. This error may be minimised by adding an inactive oil, *e.g.* arachis oil, so that the total concentration of oil in the reaction mixture is about 2 per cent.

Since such a solution is more convenient in use than undiluted AsCl_3 , it is preferable for general work, except in the rare cases where an extremely low result requires confirmation by the AsCl_3 reagent (Rosenheim and Webster, 1927; see also Wilson, 1927). This lack of sensitiveness in the antimony reagent is clearly due to the dilution with chloroform of both the oil and the reagent.

“(b) **SOLUTION OF OIL.**—To be prepared on the day when required, with anhydrous chloroform. Strength to be adjusted so as to keep the amount to be added between 0.1 and 0.3 c.c. This to be run into the cell already in position in the tintometer, and containing the reagent. To be mixed immediately by stirring with a clean, dry glass rod, and time of mixing noted.

“(c) **TINTOMETER READINGS.**—To be taken in Lovibond blue units, 30 seconds after mixing.

“(d) **TEMPERATURE.**—To be kept at about 16° C. Divergence of more than 1 degree from this to be noted, and a correction applied.

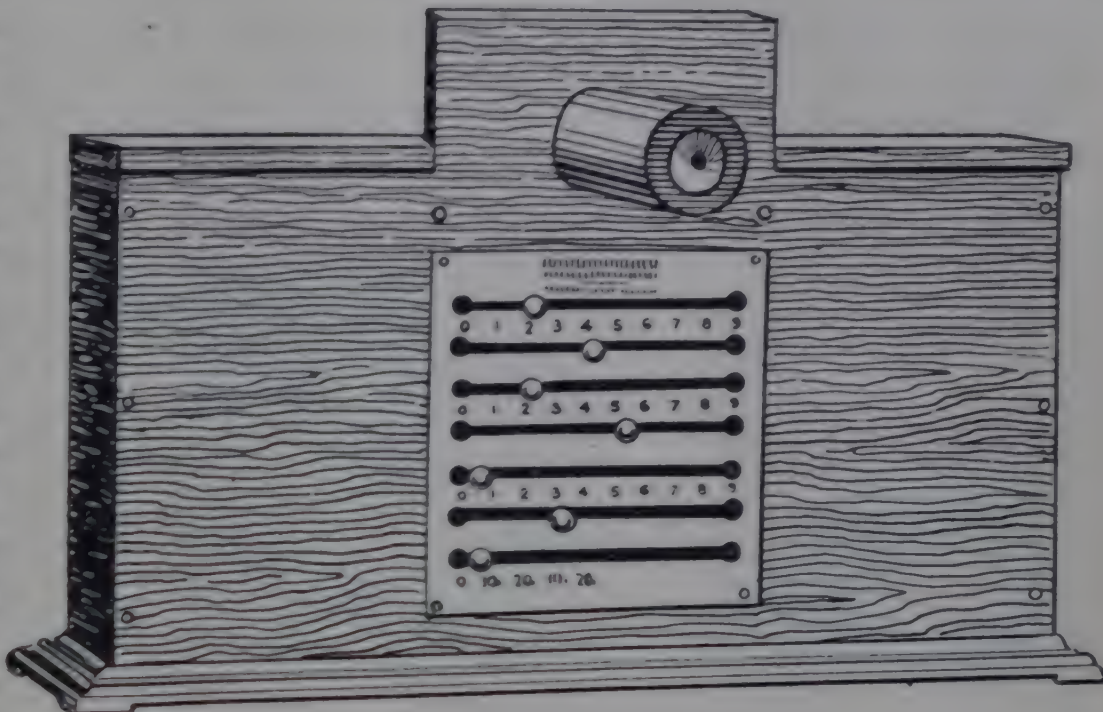
“(e) **CONDITIONS** to be adjusted so that reading of blue units is below 18, using $\frac{1}{2}$ -inch cell.”

This reaction is characterised by an absorption band at about 610 $\mu\mu$.

In both the above reactions the use of a new colorimeter, based on the Lovibond Colour System, and designed by Rosenheim and Schuster (1927), is recommended.

The standard glasses are arranged in frames containing 10 each of the red, yellow, and blue units, in such a way that they can be rapidly moved horizontally behind a window below the cell containing the coloured solution to be matched. Other frames contain tenths of

three glasses having a tint of 10 units of blue, yellow, and red respectively. By using the different coloured glasses, an accurate match with any particular coloured solution can be made, but only the essential colour value of the reaction itself is taken for the purpose of calculation, *i.e.* blue in the case of vitamin A with AsCl_3 and SbCl_3 . In the arsenic chloride test, the oil to be tested is measured by means of a capillary micro-pipette delivering 20 mgrms. of oil into a test-tube of clear, white glass of 10 mm. diameter. One c.c. of pure AsCl_3 is delivered from a standard 10 c.c. burette into the test tube and a reading taken in the test-tube immediately (time limit 30 seconds).



Five consecutive tests are made, the results being expressed in standard units of blue.

Gutzeit (1927) has suggested a more stable modification of the antimony chloride reagent by dissolving 20 grms. SbCl_3 in 50 c.c. chloroform and adding 10 c.c. of chloroform containing 0.2 gram hydroxylamine hydrochloride and 0.5 gram AsCl_3 . On using this reagent on various cod-liver oils it was noted that a blue colour was characteristic of vitamin A, whereas a red colour, often interfering with the blue and resulting in purple, was characteristic of vitamin D; it is given by irradiated cholesterol. Both of these colour intensities were parallel to their respective vitamin potencies when tested by physiological assay.

In the colour tests for vitamin A, employing sulphuric acid with arsenic or antimony trichlorides, the initial blue colour has been adopted by many workers as indicative of vitamin content rather than the red colour which develops on standing. Wokes (1928) has investigated the action of the above "vitamin" reagents and other reagents on a number of sterols and their derivatives. In many cases a red colour only is obtained, but a blue colour was obtained with sterols under the following conditions:

- (1) With antimony pentachloride;
- (2) with acetic anhydride and concentrated sulphuric acid;
- (3) with "vitamin" reagents on ergosterol in high initial concentration;
- (4) with "vitamin" reagents on sterols treated with "oxidising" agents, formaldehyde or acetic anhydride.

These sterol colours are more stable, however, than the "vitamin" colours, and may take a day or more to change to red or red-brown, whereas the "vitamin" blue colour usually disappears within a few minutes. It was found, however, that irradiation of the sterols may cause the colours to be more transient, while, on the other hand, under certain conditions the "vitamin" blue may persist for nearly an hour.

Rosenheim (1927) has also investigated some sterol colour reactions in their relation to vitamin A.

Cholesterol in chloroform solution when subjected to mild oxidation with benzoyl peroxide or nascent formaldehyde gives rise to a chromogenic substance which produces with AsCl_3 a blue colour indistinguishable from that given by cod-liver oil. Cholic acid similarly

treated also gives a blue colour with AsCl_3 . These reactions suggest that oxidative changes of the sterol molecule may be concerned in the formation of vitamin A from sterols. When purified cholesterol was added to vegetable or animal oils, which do not themselves react with AsCl_3 , the production of the blue colour was completely inhibited.

Rosenheim concludes that the chromogen artificially produced from cholesterol is not identical with the chromogen of cod-liver oil.

(3) **Fearon's Pyrogallol Test.**—According to Moore (1929) the specificity of the colour reactions for vitamin A still remains a matter of doubt.

It is pointed out also by Jones and co-workers (1929) that it will not be possible to apply this test successfully until definite knowledge is obtained of the constituents in the oil, which are related to the development of the blue colour, and the manner in which these substances are affected qualitatively and quantitatively by the conditions under which the oil is examined.

METHOD OF TESTING.—One or two drops of the oil, or ethereal solution of the substance to be examined, are placed in a dry flask, and a few dry crystals of pyrogallol added, and then 5 c.c. of 12 per cent. trichloroacetic acid. On shaking the contents well a bluish-pink colour slowly develops, changing to deep rose, which is stable, and will remain for days if the flask be properly closed.

Chemical and biological evidence shows, however, that this reaction has no relation to vitamin A.

Willimot and Wokes (1927) carried out a series of experiments in which the destruction of the vitamin A of cod-liver oil samples was not followed by a parallel decrease in the intensity of the colour produced by the reagents. The rose-red colour was still given by a sample which had been treated with concentrated sulphuric acid and which was proved by feeding experiments to have lost its vitamin A; the arsenic and antimony trichloride colour tests on the "sulphonated" oil were negative. Similar results were obtained with another sample of oil inactivated by prolonged exposure to ultra-violet light.

Rosenheim and Webster (1926) found that fish-liver oils alone give the Fearon reaction; it was given by samples of fish oil that were proved by feeding experiments not to contain vitamin A., and, on the other hand, the reaction was not given by a sample of pig's liver fat which contained abundance of vitamin A as measured by feeding experiments. They suggest that the chromogen of the reaction is associated with aldehydic oxidation products of unsaturated fatty acids, of the type of clupanodonic acid.

(4) **Monomolybdophosphotungstic Acid (Bezssonoff).**—Bezssonoff (1929, 1930) has described a reaction giving a blue-violet colour with monomolybdophosphotungstic acid, for which he claims greater specificity than the antimony chloride test. He suggests that the reacting body may be, if not vitamin A itself, a substance more constantly associated with vitamin A than the chromogen which he believes to be the active agent in the antimony chloride reaction.

(d) **APPLICATION OF COLOUR TESTS TO THE VITAMIN A CONTENT OF NATURAL PRODUCTS**
—**Cod-Liver Oil.**—The validity of the colorimetric method for estimation of vitamin A in cod-liver oil was tested under the auspices of the League of Nations (1925). Specimens of cod-liver oil were supplied to different laboratories, one of which (the National Institute for Medical Research—Dr. Rosenheim) tested them colorimetrically, using the arsenic and antimony chloride methods, and the others biologically.

The following biological method was adopted :

- " 1. Young rats of weight 40 to 50 grms., 20 to 30 days old, to be used.
- " 2. The number of control animals to be equal to that in each group receiving oil.
- " 3. The animals to be fed on artificial diet devoid of fat-soluble vitamin until growth ceases, and excess of antirachitic vitamin (in form of irradiation, irradiated foodstuffs, or

irradiated cholesterol) to be supplied during the whole or latter part of this pre-observation period.

"4. The material to be tested to be added to the diet when growth has ceased and the weight of the animal has become constant or is beginning to decline.

"5. The animals to be observed during a further period of four weeks, during which time the antirachitic vitamin should be supplied.

"6. Comparison to be instituted between the doses of the various oils required to restore the same rate of growth.

"7. Not less than three animals to be used for each dose of oil. The oil to be administered separately to each animal. The diluent to be some inert solvent, whose inactivity should be controlled by the behaviour of control animals."

The following table shows the results obtained by the different workers :

	Laboratory.	Observer.	Method.	Vit. D. supplied as—	Cod-Liver Oils.							Remarks.
					1	194	202	97	140	114	82	
1	Nat. Inst. for Med. Research.	Dr. Rosenheim.	C.	—	100	88	45	34	34	20	0	Expressed in units of blue (Lovibond) mean of values given by AsCl_3 and SbCl_3 reactions, using 1 c.c. reagent and 20 mgrms. oil.
2	Univ. of Sheffield Pharmacolog. Dept.	Prof. E. Mellanby.	B.	Irradiated cholesterol.	++++	+++	++	++	++	+	0	Graded by— Comparison of doses required to restore same rate of growth: 82 has toxic properties.
3	Univ. Coll. Lond. Biochem. Dept.	Prof. J. C. Drummond.	B.	"		++	+++	++	+	±	0	Comparison of rate of growth on 10 mgrms. doses.
4	King's College for Women, Physiol. Dept.	Prof. Mottram and Dr. G. Hartwell.	B.	"		+++	++	++	++	+	0	Comparison of rate of growth, general condition and, where possible, post-mortem examinations on 5 mgrms., 10 mgrms., 15 mgrms., and 20 mgrms. doses.
5	Lister Inst. Dept. of Exp. Pathology (Prof. Sir C. Martin).	Miss K. M. Soames and Miss J. Leigh Clare.	B.	Irradiated hardened cotton-seed oil.	+++	++	+++	++	++	+	±	Comparison of rate of growth on 20 mgrms. doses (Lister standard cod-liver oil = +++).
	Univ. of Oslo.	Prof. Poulsson.	B.	Not supplied.		100 (0.5 to 0.75)	66 (0.75 to 1.0)	30 (2)	66 (0.5 to 1.25)	30 (2)		Comparison of smallest growth-promoting dose (in mgrm.).

C. = Colorimetric.

B. = Biological.

As a general result of the test the conclusion was drawn that the colorimetric method of Rosenheim and Drummond afforded information consistent with that derived from the biological tests, but the committee considered that before any definite statement as to the general validity of the colorimetric method for assay of vitamin A could be drawn, tests should be made with substances other than cod-liver oil containing vitamin A, such as butter, palm oil, cereal oils. Drummond and Baker (1929) have gone so far as to suggest that the vitamin may be assayed more accurately by colorimetric than by biological methods.

In other quarters, however, the specificity of the colour reactions has been questioned. On the one side Steudel and Peiser (1923) have stated that the "vitamin" reaction is given by inactive crude cholesterol, while in the opposite direction Steudel (1929) has also encountered a substance which, although active in doses of 1 gram, produced no colour reaction. These criticisms, although indicating certain difficulties which might arise in the application of the colorimetric technique, can be readily answered on theoretical grounds. For, in the first instance, chromogens derived from cholesterol may usually be readily distinguished from the vitamin chromogen by their inability to produce colorations when dissolved in natural oils; in the other direction very weak sources of the vitamin may produce colorations so faint as to escape observation unless the test is carried out on the unsaponifiable fraction rather than on the whole oil. Further criticism of the specificity of the reaction has been put

forward by Hawk (1929), who has claimed that the chromogenic value of cod-liver oil may actually increase in samples exposed to climatic conditions calculated to cause destruction of the vitamin. Jones, Briad, Arzoomanian, and Christiansen (1929) have also reached a similar conclusion, having found that in certain samples of cod-liver oil the colorimetric assay might exceed the biological assay by as much as 600 per cent.

The following table (according to Drummond) shows the comparison of the vitamin A content of the three oils A, B, and C, tested by the spectro-photometer, Drummond's colour reaction, the absorption band, and the biological test respectively :

					A	B	C
Spectro-photometer	1	1.77	3.13
Drummond's reaction	1	1.9	2.9
328 band	1	1.57	3.21
Animal experiment	1	1.4	2.3

Recent experiments by Rosenheim and Webster (1929) show that the band at 320–325 cannot by itself be taken as a criterion for vitamin A. On examining spectrographically dihydro-ergosterol, a sterol with four double bands prepared from ergosterol, it was found to exhibit intense absorption in exactly the same region as that claimed to be specific for vitamin A. The free sterol, as well as its acetate and peroxide, proved to be devoid of growth-promoting properties when fed to rats on a vitamin A-free diet in doses of from 0.001 mgrm. to 1 mgrm. per day, vitamin D being supplied as irradiated ergosterol. These substances give no blue colour with arsenic or antimony trichloride.

EFFECT OF OTHER FACTORS ON COD-LIVER OIL AS MEASURED BY COLOUR TESTS.

(1) **Heat and Oxidation.**—Wokes and Willimott (1927) followed by colour tests the progressive destruction of vitamin A by aeration at different temperatures between 85° C. and 125° C. Tests with concentrated sulphuric acid, phosphorous pentoxide, arsenic trichloride, and antimony trichloride gave qualitative agreement, the two latter being more sensitive. Curves were given showing the rate of destruction of vitamin A by aeration at temperatures between 85° C. and 125° C., using arsenic trichloride and antimony trichloride for quantitative determinations. Preliminary tests indicate that the destruction of the vitamin during aeration may be due to the formation of volatile organic peroxides. The time required for the complete destruction of vitamin A in the sample of cod-liver oil by aeration and heat was as follows : 105 minutes at 88° ; 75 minutes at 98° ; 50 minutes at 108° ; 35 minutes at 118° ; and 30 minutes at 125°.

(2) **Light.**—Evers (1929) thinks that the variation as shown by colour tests in the vitamin A content of cod-liver oil, which has been stored, is not due to the ultra-violet rays in light. He found that exposure to air brought about the change in the test, though not so rapidly as sunlight.

(3) **Irradiation.**—It was found by Wokes and Willimott that the destruction of vitamin A in the presence of actinic rays went on after the irradiation had ceased, so that an oil in which only 3 per cent. of vitamin A was destroyed by this method was found three months later to have lost 25 per cent.

(4) **Pigments.**—Pigments which might interfere with the colour reactions must be removed by absorption before substances such as butter, cheese, orange rind oil, spinach, carrots, etc., can be tested for their vitamin A content.

Willimott and Wokes (1927) found it possible to remove these pigments in foodstuffs such as butter fat, egg-yolk fat, etc., by several hours' shaking of a petroleum ether solution with good charcoal (10 grms. to 100 grms. fat), and vitamin A did not appear to be absorbed to

any great extent by the process. This permitted the successful application of colour tests to a series of cod-liver oils and derived products, to butters and margarines, egg-yolk fat, suet, lard, dried milks, citrus oils and juice, and to extracts of spinach, carrots, and yellow maize.

In the case of butter and cheese, Willimott and Wokes (1927) found that annatto, which is used as a colouring agent, gave a transient blue colour with the vitamin reagents, due probably to its constituent bixin. The results obtained by these workers on fresh orange rind oil (1927) after discoloration confirm Morgan's conclusions (1923) that it is one of the most potent sources of vitamin A in the vegetable kingdom. Spinach leaves were extracted by acetone ether and decolorised by norit, as were also carrots and yellow maize.

The following table, taken from the *Lancet* (2nd July 1927), shows the results of the colour tests on various foodstuffs compared with the biological test:

COLOUR TESTS FOR VITAMIN A IN FOODSTUFFS AND NATURAL PRODUCTS.

Material Tested.	Colour Tests (a).				Vitamin A Content by Feeding Experiment (b)
	Conc. H_2SO_4 .	P_2O_5 .	AsCl_3 .	SbCl_3 in CHCl_3 .	
Dairy butter, Danish (g) ...	?	?	+	+	+
After adsorption of pigment (g) ...	?	?	+	+	+
Vegetable margarine (h) ...	—	—	—	—	—
With annatto (i) ...	+	?	+	+	—
After adsorption of annatto ...	—	—	—	—	—
Egg-yolk fat ...	?	?	+	+	++
Kidney beef suet (j) ...	—	—	+	+	+
Shredded suet ...	—	—	—	—	—
Lard (3 samples) ...	—	—	—	—	—
Dried milk food, roller process (k) ...	?	?	+	+	+
Spray process (k) ...	?	?	±	±	—
Orange rind oil ...	?	?	++	++	+++
Lemon rind oil ...	—	—	—	—	—
Orange juice extract ...	?	?	+	+	+
Spinach extract ...	?	?	++	++	+++
Carrots extract ...	?	?	+	+	+
Yellow maize extract ...	?	?	+	+	+

(5) **Carotin.**—Recently Euler and co-workers (1928, 1929) have indicated certain other objections to the general application of the colorimetric method of estimation. Different "carotinoids" have been found to give different reactions with varying intensity and shade, and no correlation has been found between the intensities of the colorations produced and the activity of the various carotinoids. Certain carotinoids—xanthophyll, lycopin and crocetin, bixin and capsanthin—have, in fact, been found to produce colorations, although biologically inactive. These workers also state (1930) that all the carotinoids which they have examined give varying degrees of blue with antimony trichloride, but only one—diodocarotin—has growth-promoting properties. Colorimetric data can, therefore, only be used as a basis of comparison when they are given by the same carotinoid or by a mixture of these substances in constant proportions.

Bezssonoff (1929) suggests that the easiest method to eliminate the influence of lipochromes is to use solutions diluted so that their content of lipochromes is insufficient to give the colour reaction. Using his monomolybdophosphotungstic acid reagent, he brings a benzene solution of the carotins of carrots or the mixed carotins and lycopins of tomatoes to the colour of an aqueous solution of potassium bichromate (199 mm. to the litre). This solution is then diluted to one-quarter of its original strength. According to a calculation based

on the investigations of Connor (1928) the solution so obtained contains about 12 mgrms. of carotin per litre. To 2 c.c. of this solution are added 4 c.c. of monomolybdophosphotungstic acid, and if vitamin A is present a blue-violet colour diffuses into the aqueous layer, while a green colour appears in the benzene layer. The reaction is standardised by comparison with the reaction of monomolybdophosphotungstic acid with an aqueous solution of hydroquinone. Observations by Moore (1929) on the vitamin A activity of recrystallised carotin have already been described (see p. 68).

It will be recalled that the well-known reaction of cod-liver oil with antimony trichloride is characterised by an absorption band at about $610\ \mu\mu$, and until the discovery of the activity of carotin samples it seemed probable that the danger of the simulation of the test by casual inactive bodies could be ruled out by a spectroscopic confirmation of the position of the absorption bands. With highly active samples of carotin, however, the shade of colour produced is noticeably duller and more green, and is characterised by the appearance of an absorption band at $590\ \mu\mu$. Any trace of the $610\ \mu\mu$ band, if present, is too weak to be detected, although judging by the biological activity well marked adsorption at this position would be expected. Vitamin A activity cannot therefore be restricted to materials producing adsorption at a specific position.

Moore's experiments on the oxidation of carotin show that the production of a blue coloration of any shade is not necessarily a sign of vitamin A activity. Pummerer and Rebmann (1928) showed that when carotin in chloroform solution was treated in the cold with benzoyl peroxide an almost complete bleaching was effected, which was accompanied by disappearance of physiological activity. Nevertheless it was found that the chromogenic value remained unaltered, and in common with vitamin A a transient blue coloration was still given even after dissolving the reaction products in arachis oil. In this case, therefore, a colorimetric assay of vitamin activity by the tintometer method would have furnished a completely deceptive result. Subsequent spectroscopic examination disclosed that the absorption band attributable to the original carotin was no longer in evidence. Selective absorption was much less clearly defined but appeared to be mainly at about $640\ \mu\mu$.

In Moore's experiments (1929) rats receiving a rachitogenic diet (Steenbock) were given daily graded doses of a sample of carotin (M.P. 174°) for 36 days. One rat received 0.75 mgrm. daily (a huge dose), but nevertheless the liver fat showed only feeble pigmentation, but a very intense reaction was given with SbCl_3 , showing an absorption band at $610\text{--}630\ \mu\mu$ (vitamin A) and not at $590\ \mu\mu$ (carotin). In the case of the three rats receiving carotin in excess of the minimal dose (0.01, 0.1, 0.75 mgrm.), high values in blue units were obtained in the liver fat, but there was no marked increase in yellow units (carotin). The greater part of the carotin ingested therefore does not appear in the liver unchanged. It appears probable that the disappearance of the pigment and the appearance of the vitamin are connected.

On the basis of these observations, Moore concludes that materials which give no blue coloration with antimony trichloride, even after the removal of saponifiable matter, must be devoid of vitamin A activity. Materials of liver oil origin giving colour reactions characterised by absorption at $610\ \mu\mu$ may be considered active. But, on the other hand, materials which give colour reactions characterised by absorption at other positions may be either active or inactive, or mixtures of inactive and active chromogens. It is obviously not correct to estimate carotin by the use of SbCl_3 alone without spectroscopic data and yellow coloration readings. From this point of view the biological technique still remains the only satisfactory method of assay.

(6) **Presence of Vitamin D.**—The presence of vitamin D in cod-liver oil had no effect on the colour tests whilst a vitamin D concentrate gave negative results (Willimott and Wokes (1927)).

(7) **Olive Oil.**—Olive oil and oleic acid appeared in the experiments of Willimott and Wokes (1927) to exert some inhibitory action on the colour tests, since low concentrations of cod-liver oil (5 per cent.) in olive oil often could not be detected by this method. Olive oil would seem a suitable diluent for cod-liver oil, but the presence of organic peroxides may possibly gradually destroy the vitamin A.

(8) **Unsaponifiable Matter.**—According to Schmidt-Nielsen (1929) the blue colour of the antimony and arsenic trichloride tests is probably dependent on the content of unsaponifiable matter of substances accompanying the vitamin. He states that the flesh oils of fat-rich fishes, as herring, sprat, mackerel, capelin, although rich in vitamin A, never give the antimony trichloride reaction, while the liver oil of the basking shark, though rich in vitamin A, gives neither the Sb- nor the AsCl_3 test.

(9) **Variations in Technique.**—A study by Evers (1929) into the variations obtained in five different laboratories showed, in a sample of potent oil, variations from 13.2 to 16.8 units, and in a weak sample from 4.6 to 7 units. Evers suggests that a carefully defined method should be followed and allowance made for individual variations which always occur in colorimetric work.

COLOUR REACTIONS FOR VITAMIN A IN ORGANIC TISSUES.—W. H. Wilson (1927) carried out experiments with fatty extracts of liver in their relation to the antimony trichloride test.

The minced organ was allowed to stand for 24 hours in three times its volume of 94 per cent. alcohol (to facilitate drying), the residue then being strained and squeezed in a cloth. Only a negligible amount of vitamin A was lost at this stage. The residue was dried in an air oven at about 95°C ., ground to a fine powder, and allowed to stand in ether for 24 hours. The ether extract was filtered off, the ether evaporated off, and the weighed fatty residue dissolved in chloroform and tested for vitamin A by addition of 10 volumes of a 30 per cent. solution of antimony trichloride in chloroform. The standard unit value taken was a sample of cod-liver oil which when tested in 20 per cent. solution gave a colour matched by a $1/5,000$ solution of indigo carmine. Extracts from human liver gave values as high as 25, but varied within wide limits. Under conditions of disease, values as low as 0.1 were recorded, though from the results so far obtained it is regarded as premature to draw any conclusions as to the significance of the amount of vitamin A found in the liver after death in relation to the diseases causing death. The vitamin is present in the human liver at birth but not in the placenta. The extract of livers of healthy animals slaughtered for food contained 6 to $12\frac{1}{2}$ times the amount of vitamin A present in cod-liver oil. It is pointed out that if the substance giving the colour reaction with antimony trichloride is identical with vitamin A, the observations on the livers of animals used for food would indicate the value of liver as a foodstuff, especially for children.

Rosenheim and Webster (1929) have not accepted the $328 \mu\mu$ as an absolute criterion of vitamin A, and have given as one of their reasons the fact that dihydro-ergosterol exhibits a similar absorption band. Recent work by Morton, Heilbron, and Spring (1930) contests their criticism. These latter workers state that dihydro-ergosterol, while showing absorption in approximately the same region as vitamin A, exhibits four narrow bands instead of one broad one, and has only one-sixth the intensity of absorption.

(e) **PREPARATIONS OF VITAMIN A FROM SOURCES OTHER THAN COD-LIVER OIL**—(1) **Vegetables.**—The nature of the unsaponifiable fraction of the lipid matter extracted from dried spinach leaves has recently been the subject of investigation by Clenshaw and Smedley-MacLean (1929). The unsaponifiable fraction was extracted with ether, sterols were removed by digitonin, and the residue dissolved in hot alcohol. On cooling, white crystals were obtained which were identified as the hydrocarbon hentriacontane ($\text{C}_{31}\text{H}_{64}$). Cabbage leaves worked

up similarly gave the same product, together with an oxygen containing product not present in spinach. It is an interesting fact, comparing these investigations with those of Drummond on the unsaponifiable fraction of cod-liver oil, that two of the richest sources of vitamin A, namely, green leaves and fish-liver oils, both contain—(1) a highly unsaturated hydrocarbon, *e.g.* carotin and squalene; (2) products obtained by condensation and reduction of higher fatty acids, *e.g.* hentriacontane and batyl alcohol; and (3) sterols.

Preparations of vitamin A from vegetable juices have been made by Bezssonoff (1929).

These juices, extracted from green cabbage, tomato, and ripe carrots are precipitated by a concentrated aqueous solution of neutral lead acetate. After filtration and washing with water the precipitate is dried *in vacuo* at about 50° C., and then extracted in a Soxhlet apparatus with petrol ether or benzene. After a further evaporation *in vacuo* the residue is dissolved in ethylic ether and mixed with arachis oil, the ether being afterwards removed by evaporation *in vacuo*. The solution is kept in ampoules of yellow glass, and the whole procedure is carried out either in darkness or in red light.

(2) **Gallosterin.**—A combination of vitamin A with choleic acid, to which the name “gallosterin” has been given, has been prepared by Schimiza and Hata Keyama (1929). They obtained yellow crystals of the choleic acid salt of vitamin A by the addition of vitamin A to deoxycholic acid.

They state that in experiments with mice gallosterin successfully supplemented a vitamin A-free diet while deoxycholic acid did not, and that a solution of vitamin A alone made from gallosterin does not stimulate growth as completely as does the gallosterin. They conclude that the accessory portion of the compound plays some rôle in the utilisation of vitamin A.

Matsuoka (1929), however, found no appreciable vitamin A potency in gallosterin. He fed to young white rats a vitamin A-free diet to which gallosterin was added in such quantities as to give 1 mgrm., 10 mgrms., and 100 mgrms., per rat per day. Rats so fed failed to grow normally, and eventually died, having in some cases displayed symptoms of xerophthalmia.

XXII. STABILITY OF VITAMIN A.

The susceptibility of vitamin A to oxidation is a more potent factor in its stability than its reaction to heat. Its comparative stability to alkali has rendered its concentration in high degree a possibility.

(a) **OXIDATION.**—Hopkins, in 1920, showed that the vitamin A in butter was completely destroyed by heating the butter to 120° C. for 4 hours if oxygen was bubbled through the butter, although practically no destruction occurred if the butter was heated without aeration. The experiment established the important fact that this vitamin, though fairly resistant to heat, is readily destroyed by oxidation. He also observed that vitamin A disappears from butter spread in thin layers and exposed to air temperatures of 15° C. to 25° C., while Drummond and Coward (1920) found that the loss was considerable at 37° C. They concluded that the destruction observed by Zilva (1919), from the rancidity of butter which had been exposed for 8 hours in thin layers to the action of ultra-violet light, was due to oxidation, and not, as Zilva supposed, to the ultra-violet rays. The absence of vitamin A from lard is an instructive example of the conditions affecting its stability.

Drummond and co-workers (1920), in the course of their investigations, showed that although the fat of hogs, which had been suitably fed, had quite a high fat-soluble value, little of the vitamin value succeeded in reaching the consumer of the lard.

Drummond attributed this to the mode of separating and clarifying the hog fat, which involved considerable exposure to the air at temperatures up to 102° C. In cases in which the colour or flavour of the lard is unsatisfactory, the product is usually treated by the process of “blowing” or some similar method involving oxidation. To the exposure and this “blow-

ing" process may be attributed the destruction of the fat-soluble factor and the low fat-soluble content of most commercial lards.

It may be mentioned here that there is a second factor in the low vitamin A content of lard. The fat of the pig, probably because the animal fattens quickly, contains less vitamin A than that of other animals, and when a young pig is fed on a diet very deficient in vitamin A, such as toppings and whey, it may not be possible to detect that vitamin in the body fat.

Vitamin A is also destroyed when oils are "hardened" by the action of hydrogen—a process widely employed in the preparation of edible fats.

The destructive effect of oxidation on vitamin A is also suggested by experiments reported by W. C. Powick (1926), in which the vitamin A in dried egg-yolk fed to rats became inactivated when rancid fat was added to the diet.

The destruction of vitamin A was considered to be due to oxidation brought about by the peroxides present in the rancid fat. The same effect is reported by Fridericia (1924) from the action of certain fats which have been subjected to a temperature of 102°–105°, with the probable formation of peroxides.

(b) **HEAT.**—Since vitamin A is fairly resistant to heat in the absence of oxygen (it survives a temperature of 120° C. for 12 hours, and is only destroyed by 2 hours' exposure to a temperature of 200° C.), it survives most of the ordinary processes of cooking, but prolonged boiling, such as occurs in making stews, probably destroys most of it.

Pasteurisation.—According to Dutcher, Honeywell, and Dahle (1927), pasteurisation of milk as drawn from the cow contains quite appreciable quantities of oxygen in solution. It has been shown that, after milking, 13 per cent. of the gases dissolved in milk were oxygen, and it is not impossible that in the course of 30 minutes' exposure to a temperature of 140° F. this dissolved oxygen might be responsible for appreciable vitamin A destruction.

Vitamin A from vegetable sources has been found to be more stable to heat than that from animal fats and oils. Tomato juice heated for 4 hours to about 98° C., both at normal acidity (*pH* 4.2), and also in an alkaline medium (bringing it to *pH* 9.2), was found by Sherman, Quinn, Day, and Miller (1928) to show practically no destruction of vitamin A. Dried spinach leaves were also used as a comparison by the same investigators; they found that about 20 per cent. of the vitamin A in spinach, and about 33 per cent. in butter, were destroyed by heating for 4 hours at 97–99° C., under anaerobic conditions, indicating that vitamin A from this vegetable source is more stable than that in butter. Freezing has apparently little influence on the potency of vitamin A, since A. H. Smith (1922) found it present in typical samples of ice-cream to such an extent that normal growth was induced by 1 gram of the ice-cream, and ophthalmia was cured by 0.25 gram containing 25 mgrms. of butter fat.

(c) **PRESERVATION.**—Vitamin A is the easiest of the vitamins to preserve. Holmes (1922, 1924) states that it can be kept with no diminution of its curative action in cod-liver oil for 6 and even 12 months, if protected to a certain extent from the action of the air, while Poulsson (1924) asserts that cod-liver oil kept in well-corked white glass vessels for 31 years has been found active in 5 mgrms. doses. Holmes and Piggot (1926) investigated the effects upon cod-liver oil of storage at varying exposures to light.

The samples were kept for 14 to 24 months, and their vitamin A content was then tested by feeding them to rats suffering from vitamin A deficiency. Storage in a dim basement room, or in a glass case in a room so lit that sunlight could not fall on the samples, did not appreciably affect the potency of the oil. But as the light to which the bottles were exposed became more intense the vitamin A potency decreased by storage; 7 mgrms. daily of a sample exposed to sunlight daily for 16 months failed to induce any growth in the young rats, whereas less than 2 mgrms. of a sample stored in the dark induced prompt growth. In all cases the light passed through a window frame before falling on the bottles, so that the ultra-

violet portion of the spectrum could not have produced the inactivating effect. It is clear that cod-liver oil should be kept in the dark as much as possible during storage.

These results are confirmed by Drummond and Morton's recent method (1929) of testing for vitamin A. The cod-liver oils tested by them were found to lose 50 per cent. of their potency when stored for a year in the light, and 25 per cent. when stored for the same time in the dark.

Evers (1929) also recommends the storage of cod-liver oil in amber bottles.

A preservative action of hydroquinone on the vitamin A content of stored cod-liver oil and milk fat has been reported by Huston, Lightbody, and Ball (1928). The hydroquinone apparently protects the fats from chemical decomposition, and has a definite anti-oxygenic effect on the vitamin A.

(d) **ULTRA-VIOLET RAYS.**—The conclusions to be drawn from Zilva's experiments (1919) that the destructive action of ultra-violet rays is, in fact, merely the result of a process of oxidation seems to be generally accepted, and Spinka's (1924) statement that butter treated by ultra-violet rays develops a toxic action has been shown by Zilva to be insufficiently demonstrated. Whether the destructive action in milk is due to the action of the ultra-violet rays themselves or to oxidation remains unproven, though Evers' experiments (1929) indicate that destruction goes on even when the ultra-violet rays are cut off. That milk which has undergone irradiation does lose its vitamin A potency has been shown by the experiments of Titus, Hughes, Henshaw, and Fitch (1926).

Two lots of chicks were irradiated with a quartz mercury-vapour lamp for 15 minutes per day. The same ration was given to both lots, except that the 5 c.c. of fresh whole milk supplied in one lot was irradiated and in the other non-irradiated. The milk in a sheet-iron pan was irradiated by subjecting to the rays of the quartz mercury-vapour lamp, placed 18 to 20 inches above the surface, agitating the milk. At the end of 7 weeks the chicks, which had the non-irradiated milk, showed no vitamin A deficiency. One chick remained in the other lot apparently able to survive the unfavourable conditions, the other chicks having died with vitamin A deficiency.

This result is contradicted by the experiments of Supplee and Dow (1927), who found no evidence for destruction of vitamin A in samples of winter milk, both dried and undried, when irradiated for a short time with the mercury-vapour lamp.

It is supported, however, by the findings of Peacock (1926) in samples of cod-liver oil with regard to its loss of fluorescence under ultra-violet light illumination. The change was found to be due to the exposure of the oil to light, and this phenomenon, called "delumination," was caused by sunshine, by a carbon-arc lamp, a mercury-vapour lamp, and a fullolite gas-filled lamp.

After several months of storage in the dark, "deluminated" oil recovered some of its fluorescence. The colour reaction for vitamin A, devised by Rosenheim and Drummond (1925), was applied to samples of the oil, and it was found that the deluminated oil gave a completely negative reaction. Feeding tests on animals confirmed the deduction that the oil that had lost its fluorescence had lost its vitamin activity. The oil that had regained some of its fluorescence after previous "delumination," by storage in the dark, still failed to give the colour reaction for vitamin A. It appears, therefore, that the action of light on cod-liver oil is a complex one which is only partly reversible.

Drummond (1927) has also suggested the destruction of vitamin A in irradiated milk. Steenbock, Riising, *et al.* (1929), in their experiments on the irradiation of cereals, found that vitamin A was labile to ultra-violet radiation, but evidence of its destruction in cereals can probably not be obtained with such an exposure as is necessary to secure maximum antirachitic activation.

Recent experiments in the irradiation of milk to increase its vitamin D potency have

resulted in methods which obviate to a great extent the destruction of its vitamin A content. (See p. 163.)

(e) **ALKALIES.**—Saponification, even with boiling alcoholic potassium hydroxide, does not appear to destroy vitamin A if the conditions are such as to avoid oxidation. Drummond has achieved these conditions by carrying out the hydrolysis in an atmosphere of nitrogen.

(f) **ACIDS.**—Luce and Maclean (1925) have found that hydrolysis by boiling with normal hydrochloric acid for 2 hours causes a marked destruction of vitamin A.

(g) **FERROUS SULPHATE.**—Vitamin A, like vitamin E, though later and in lesser degree, is destroyed by the oxidative decomposition of butter fat under the "stimulus" of ferrous iron.

Simmonds, Becker, and McCollum (1927) have found that ferrous sulphate, added to a diet rich in vitamin A, inhibits its action, and induces a "salt ophthalmia" similar to that which occurs in a vitamin A-deficient diet.

(h) **FINELY DIVIDED SOLIDS.**—A destruction of vitamin A by dry powders is indicated by the experiments of Marcus (1930). He found that when concentrate of vitamin A from cod-liver oil was mixed with dry powders such as the lactose granulation used in pharmacy, calcium carbonate, sodium bisulphite, magnesium oxide, magnesium citrate, ferric oxide, ferrous sulphate, iron powder, calcium hypophosphite, sodium hypophosphite, sodium pyrophosphite, hydroquinone, charcoal, or the U.S.P. basal ration for vitamin A, destruction of vitamin A resulted in every case, as determined, for the most part, by the antimony trichloride colour test. With Nuchar, 90 per cent. destruction occurred in a few hours; 100 per cent. in 15 days with iron powder, ferric oxide, and others; 95 per cent. in 8 days with lactose granulation, etc.

The experiments suggest that the destruction of the vitamin is due either to oxidation by the oxygen adsorbed on the large surface of the powders, or to polymerisation. It will be seen that vitamin A is in general a stable vitamin and can be relied upon to retain its virtue, despite the culinary processes of civilised living. The increasing tendency in France, however, to use domestic autoclaves for cooking food must be considered detrimental to its vitamin A content, since the temperature in these vessels reaches 140° C.

XXIII. PHYSIOLOGICAL EFFECTS OF VITAMIN A DEFICIENCY.

The outstanding effects of a definite experimental deficiency of vitamin A are lack of growth and affections of the eye leading to a generalised ophthalmia. Besides these there are specific tissue changes, including epithelial hypertrophy and hyperplasia, a characteristic disturbance of reproductive function, and various metabolic changes. Recent studies have also ascribed to vitamin A a peculiar rôle in resistance to infection, so that it has even been termed the "anti-infective vitamin."

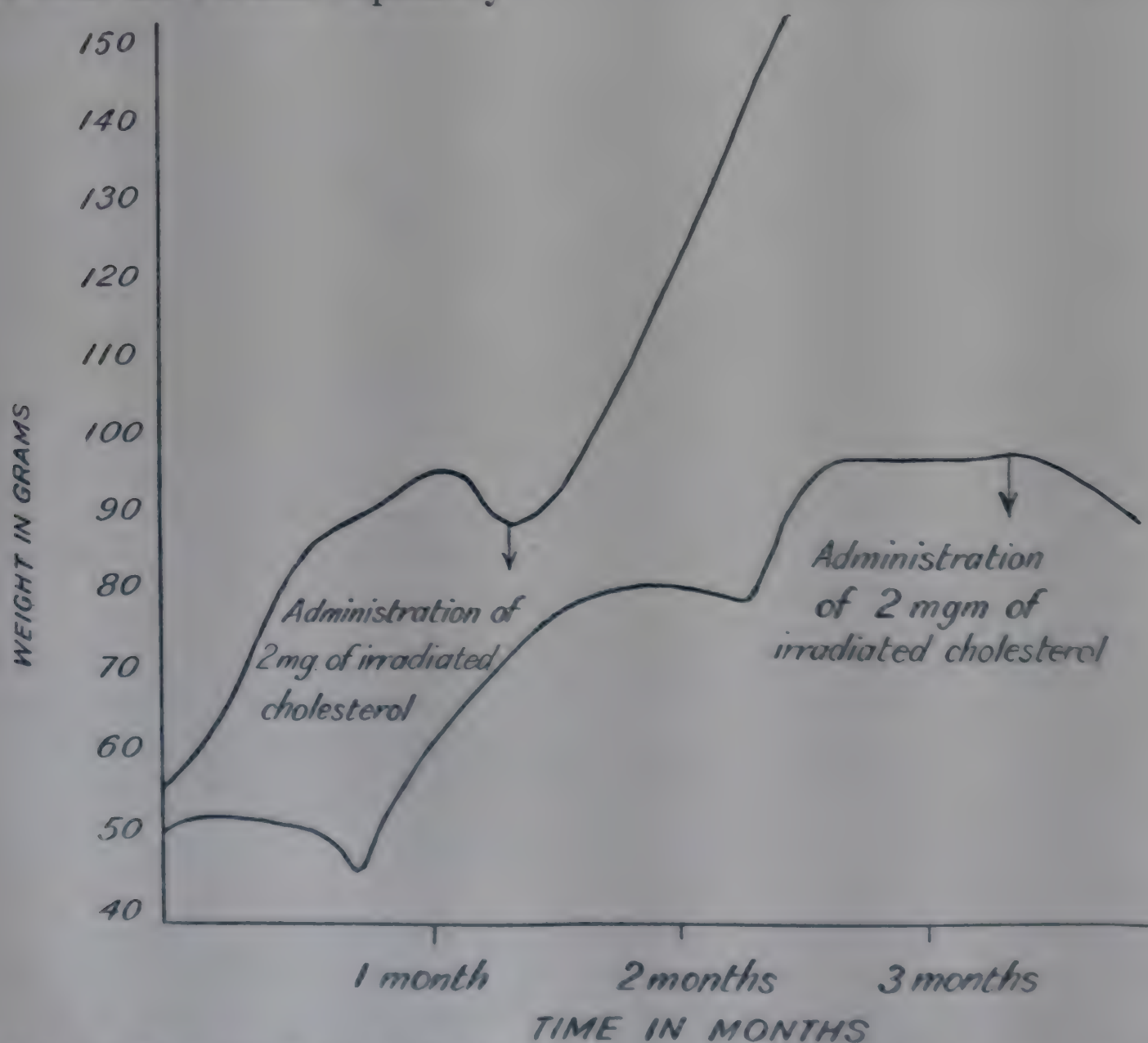
(a) **PRE-DEFICIENCY IN AVITAMINOSIS A.**—A pre-deficiency state is described by Mouriquand, Rollet, and Chaix (1930), in which conditions recognisable as due to a deficiency of vitamin A precede the onset of clinically definable symptoms. They state that it has been found possible, in the case of rats, to detect ulceration of the cornea microscopically 20 days after commencing a vitamin A-deficient diet, and 20 days before the onset of clinically recognisable symptoms of xerophthalmia. The administration of cod-liver oil clears up the ulceration in a few days.

(b) **INFLUENCE ON GROWTH.**—The growth factor in vitamin A is much more potent in the case of the growing animal than the adult. In the young animal deprivation of vitamin A results in a cessation of growth, which can be restored by administration of substances containing the vitamin. Until McCollum and his co-workers (1922) first definitely demonstrated that vitamin A was a substance quite distinct from the antirachitic factor, or vitamin D, this resumption of growth was taken as a criterion of the presence and amount of vitamin A

in the diet. Steenbock and his colleagues (1923, 1924), however, showed that the cessation of growth in animals on a standard vitamin A-deficiency diet might, in some cases, be due not only to deficiency of vitamin A, but also to a co-existing deficiency of vitamin D, and their results were confirmed by other workers.

Steenbock, Nelson, and Black (1924), administering vitamin D separately, either in the form of cod-liver oil in which the vitamin A had been destroyed by oxidation, or by irradiating their experimental animals, found that the animals so receiving vitamin D showed more growth than the controls, but developed ophthalmia at about the same time. Drummond, Coward, and Handy (1925) arrived at the same conclusion by a different method. They found that the administration of irradiated cholesterol produced resumption of growth in animals which had ceased growing on a vitamin A-deficient diet, but that the resumption only took place in animals which had received the deficient diet for a short time; after periods of 3 to 4 months, growth could not be restored. Since a small amount of cod-liver oil resulted in a prompt and steady increase in weight, they were led to the conclusion that the initial cessation of growth was due to the absence of vitamin D at a time when the animal had not expended its reserves of vitamin A, and that the real vitamin A deficiency varies with the previous feeding of the animal and depends upon the exhaustion of its bodily stores.

The following table illustrates the response to administration of vitamin D after periods of 4 to 5 weeks and 3 months respectively:



It will be seen that growth varies with the amount of vitamin A in the previous diet, and therefore presumably with the amount of vitamin A which the rats had been able to store in their tissues.

Sherman and Burtis (1928) have carried out experiments bearing upon the nutritional rôle of vitamin A in relation to growth and health, with special reference to the importance of this factor in the food of children, and have shown that the level of intake of vitamin A (and perhaps certain other of the chemical factors in nutrition) during early life may markedly influence subsequent susceptibility to infection.

Two large groups of rats were taken when 4 weeks of age, one reared up to that time on an adequate ration with a safe margin of vitamin A, the other group reared on a similar complete food but with less of the fat-soluble factor. All of these animals were then depleted of the vitamin A which they had stored by being given for a month a food free from this factor. Both groups were then fed for 8 weeks on a diet containing a limited concentration of vitamin A. At the end of the experiment autopsies were made, and the incidence of infection was determined in all the rats. In the first group this was 25 per cent.; in the second, 75 per cent. The only difference between the two groups was the dietary treatment of the mother and young up to the time of weaning, one series having plenty of vitamin A, while the other was somewhat limited in its supply. They have concluded from the investigation that a relative deficiency of vitamin A in infancy may exert a lasting deleterious effect upon the organism.

(c) **XEROPHTHALMIA.**—The eye disease, which is considered a more or less specific sign of vitamin A deficiency, has been called xerophthalmia by some workers, keratomalacia by others, while Osborne and Mendel (1921) preferred the generic term ophthalmia. Xerophthalmia is preferred by those workers, such as Mori (1922), who have found the essential lesion to consist in a xerosis or dryness of the corneal and conjunctival tissues, while Goldschmidt (1915), considering the disease in rats to be identical with keratomalacia in man, and Stephenson and Clark (1920), laying stress upon the appearance of the secondary symptoms due to bacterial invasion, prefer the term keratomalacia.

The incidence of the disease is variable, depending on the extent of the vitamin A deficiency and the age of the animal. In children the characteristic eye lesion was at first believed to be due to fat starvation, Mori (1904) having observed over 1400 cases in children of 2 to 5 years affected with xerosis and keratomalacia, often terminating in blindness, and Bloch (1921) 40 cases of necrosis of the cornea with ulceration in children fed on nearly fat-free separator milk.

In Malaya the disease is confined to the labouring classes, and is usually associated with infections of other kinds, such as dysentery, prolonged fevers in adults, helminth infections, and naso-pharyngeal catarrh in children, and "Viswalingam arthritis," the disease due to deficiency of fat-soluble vitamins in the diet, and to the lack of observance of the habit of rubbing oil frequently over the body.

The fully developed disease, as described by Holm (1922, 1925), consists of a rapid development of extensive ulceration, with a purulent conjunctival secretion, followed by perforation and ophthalmia. The early lesions have been minutely investigated by Mori (1922), who found two distinct and characteristic changes at the onset of the disease. One of these is the cornification of the outer layer of the epithelial cells of the conjunctiva, and the other the formation of the granules of keratohyalin in the cytoplasm of the outer layer of these cells.

These two changes had already been shown by Leber (1883) to be caused by dryness or xerosis of the conjunctiva. As the disease advances they spread to the fornix and the conjunctival palpebræ, constituting a keratosis in the pathological sense. According to Pillat (1929), this stage, xerosis epithelialis conjunctivæ, appears in four clinical forms—Bitot's spots, irregular xerosis of large parts of the conjunctiva, loss of lustre, and wrinkling of this membrane.

According to Treacher Collins (1930), the Harderian glands, whose mucous secretion

preserves the corneal epithelium from keratinisation in animals in which it is present, becomes atrophied in vitamin A deficiency and ceases to function. In human beings the mucous secreting cells of the conjunctiva replace the Harderian gland, and in vitamin A deficiency these atrophy, leading to the same effect of keratinisation of the cornea and conjunctiva.

Necrosis of the corneal epithelium also takes place on account of the dryness in the nutrition of the cornea. Two forms may be distinguished clinically, pre-xerosis and real white xerosis of the cornea. The three main symptoms of the former are a slight loss of lustre of the cornea, reduced sensibility, and the finding of *B. xerosis* in the degenerated epithelial cells. Real corneal xerosis presents three pictures: White crescents within the upper or lower circumference of the cornea; irregular whitish plaques in connection with the limbus; and isolated islands in the centre of the cornea, which are not connected with the limbus.

The ulceration of the cornea is a secondary change, due to bacterial invasion. The bacteria concerned in the destructive change are, according to Stephenson and Clark (1920), not specific; pneumococci were often but not invariably present, the flora including *Staphylococcus albus* and *aureus*, diphtheroid bacilli, *Bacillus subtilis*, etc.

Sampaolesi (1927) suggests that the diphtheroid isolated by him from the conjunctival sacs of white rats fed on an unbalanced diet was possibly a factor in production of avitamin xerophthalmia, although an identical diphtheroid was sometimes found in eyes of normal white rats.

Findlay (1924) has shown that the onset of keratomalacia is coincident with a fall in the lysozyme content (that is the bacteriolytic ferment described by Fleming (1922) as occurring normally in tissues and secretions) of the tears, and that the onset could be prevented by the instillation of human tears into the animal's eyes, whereas the instillation of normal salt solution had no such protective action. S. J. Cantor (1927) has stated that "the earliest chronic keratomalacial change may be an alteration in the shape of the cornea, leading to errors which can in many cases be regarded as due to fat-soluble vitamin deficiency." Other symptoms frequently encountered are pigmentation of the conjunctiva, meibomitis, blepharitis, and hordeolum, decrease of the lachrymal fluid, oedema and puffiness of the lids, and comedones near the eyes. All these symptoms disappear when proper nourishment is given or treatment with cod-liver oil is undertaken.

"Salt Ophthalmia."—McCollum and co-workers reported in 1922 and 1925 the occurrence of xerophthalmia in rats fed on a diet containing 5 per cent. of butter fat but also containing 0.2 per cent. of ferrous sulphate, while those on a diet of the same vitamin A content, but containing ferric citrate instead of ferrous sulphate, remained normal. Mori (1922) described the pathologic changes of the ophthalmia produced by this diet, with ulceration and perforation of the eyeballs, typical of the xerophthalmia of vitamin A deficiency. The salt ophthalmia could be cured either by the replacement of ferrous sulphate by a ferric salt or by the addition of wheat germ oil to the diet.

The relation of the inorganic constituents in this respect was found to be due to the increased oxidative destruction of vitamin A in the presence of ferrous sulphate. J. H. Jones (1927) carried out experiments to prove that the time elapsing before the onset of xerophthalmia is dependent on the amount of ferrous sulphate in the diet. In the first, butter fat (5 per cent.) was freshly stirred into 50 grms. of ration (containing ferrous sulphate) every 5 days; and, in the second, the ration was made up in large amounts of 500 grms. lasting 6 to 7 weeks, and identical in composition with that in the first experiment. In the first experiment with the freshly made ration, no signs of ophthalmia were observed, even after 26 weeks, while in the second experiment, in which the vitamin A-containing butter fat was in contact with the ferrous sulphate of the ration for a much longer time, the rats developed xerophthalmia within 10 weeks, with death following in a short time.

The protective action of wheat germ oil was investigated by Matill (1927), who found that both this oil and, in lesser and variable degree, certain other vegetable oils, act as "anti-oxidisers," delaying autoxidation in fats, and thereby preventing accompanying destruction of vitamins A and E. A possible explanation of this anti-oxidising action has been suggested by Holm and co-workers (1927), in relation to the acetyl value of fats. The acetyl value of a fat is a measure of its content of hydroxyl groups, and the hydroxyl group has a very powerful retarding action. Some of the vegetable oils, and particularly wheat germ oil, which have a protective action, have relatively higher acetyl values than the animal fats and oils, particularly lard and cod-liver oil.

(d) **HEMERALOPIA.**—Functional night blindness is commonly considered to be associated with vitamin A deficiency, though it has also been found in conjunction with scurvy and beriberi, the latter according to Aykroyd (1928), especially in Newfoundland, where the popular diet is deficient in both water-soluble and fat-soluble vitamins. It has been found by Bloch (1926) associated with xerosis of the conjunctiva and keratomalacia and readily cured by food containing a high percentage of vitamin A.

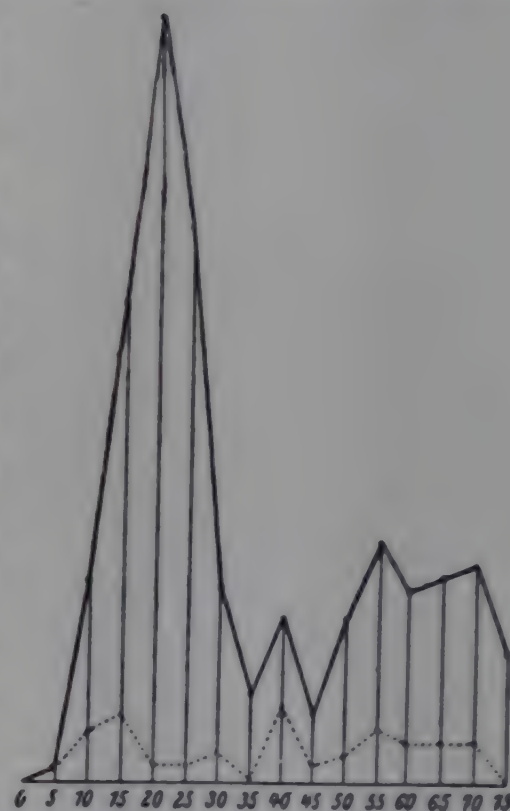
Pillat (1929), however, states that it is unaccompanied by positive eye-ground findings. The incidence of this complaint has a seasonal variation which corresponds to the deficiency of the average diet in fat-soluble vitamins, that is to say, more cases occur in the winter than in the summer months. The two sexes are affected equally up to the age of puberty, but from this time onwards men are much more frequently affected than women, until after middle life, when the difference between the numbers of cases in the two sexes becomes less marked.

H. Abels (1927) offers as an explanation of these differences the fact that women have far greater stores of subcutaneous fat than men, and in this fat a reserve supply of vitamin A is laid up in the summer which can be drawn upon in the winter and thus supply any deficiency in the diet. During pregnancy women become more liable to develop night blindness, for their stores of vitamin A are being depleted to supply the needs of the growing foetus.

Aykroyd (1930) points out, however, that strong sunlight also plays a part in its causation. He observed in Newfoundland and Labrador that men exposed for long periods to sunlight in open boats were the chief sufferers, while women and children were rarely attacked by it. Fridericia and Holm (1925) and also Treacher Collins (1930) have found that in rats the inability to see in a dim light produced by a vitamin A-deficient diet was accompanied by a marked delay in the regeneration of the visual purple (Rhodopsin), and Aykroyd considers that this delay would account for the observed clinical facts in hemeralopia.

Blegvad has recorded the interesting case of a man who suffered from cancer of the liver and perhaps pancreas, and was therefore presumably unable to absorb an adequate supply of vitamin A from his food, with a consequent development of xerosis of the conjunctivæ and severe hemeralopia. He was given subcutaneous injections of a concentrated preparation of vitamin A extracted from cod-liver oil by Professor Poulsson; after 6 injections the patient could see to walk in the streets in the evening and even read in dim light.

(e) **SPECIFIC TISSUE CHANGES**—(1) **Metaplasia and Hyperplasia.**—The first to draw



Hemeralopia frequency by age and sex. Heavy line represents males; dotted line, female. The abscissae = age; the ordinate = number of cases.

[Reproduced from the *Münchener Medizinische Wochenschrift*, according to Birnbacher (1928).]

attention to the changes in epithelium induced by diets deficient in vitamin A was Mori in 1922. He described them in the larynx, trachea, and ducts of many glands, including the meibomian, submaxillary, sublingual, and parotid glands of rats. Mori's work was confirmed and extended by Wolbach and Howe (1925), who examined histologically the tissues of rats fed on a diet composed of casein, starch, salt mixture, lard, and yeast. The changes found consisted in the substitution of keratinising epithelium for normal epithelium in various parts of the respiratory tract, alimentary tract, eyes and parocular glands, and genito-urinary tract; young rats developed these changes more rapidly than adults. The growth activity of the epithelium in its altered conditions was increased, as shown by the number of mitotic figures present and the formation of new blood vessels. While some of the glandular structures of the body were involved in this process, the liver, kidney, stomach, and intestines escaped. In general, the respiratory mucosa in the nares, trachea, and bronchi first exhibited this change, then the salivary glands, eye, and genito-urinary tract, and lastly the lachrymal glands and the pancreas. Though infection was commonly met with at the sites of the keratinising epithelium, the association was not constant.

Later observations (1928) by the same workers on the guinea-pig have shown that the essential pathological process is the same in a purely herbivorous animal. Lesions of the eye, such as are the rule in rats, did not occur, but a necrosis of the margin of the ears was a common feature. In contrast to the deficient rat, the guinea-pig frequently showed considerable amounts of subcutaneous and abdominal fat when advanced epithelial changes were present. Extensive keratinisation of the epithelium in the trachea, bronchi, bladder, and uterus was found, but in contrast to the findings in rats no keratinising epithelium was observed in the parocular glands or their ducts. A further distinction was that extensive keratinisation took place before pronounced atrophy of the organs concerned had taken place, and the early and intense change in the bladder and uterus of the guinea-pig was another essential difference. Of the ductless glands the thymus alone showed change, with marked atrophy and greatly enlarged Hassall's bodies. The earliest sign in the keratinising process was the presence of groups of cells deep in the epithelium having deeply staining basic cytoplasm and large nuclei, which increased by mitotic division, the growth rate being apparently augmented as judged by numerous mitotic figures and the downgrowth into supporting tissues. The observations suggested that the growth of the replacing stratified epithelium is not responsive to the regulatory factors which control normal covering epithelium.

Masaki (1927) has observed pathological changes in the mucous membrane of oral and nasal cavities of albino rats on a vitamin A-deficient diet.

Freudenthal (1927) has also found atrophy, keratinisation, and desquamation of epithelial cells in severe vitamin A deficiency, and Goldblatt and Benischek (1927) have been able to induce metaplasia of columnar, cuboidal, and transitional epithelia to the squamous keratinising type in vitamin A deficiency uncomplicated by deficiency of vitamins C and D. Twenty-three of the 26 rats on diets deficient in A vitamin only showed metaplastic changes in one or more of the following organs: trachea, large bronchi, small bronchi or bronchioles, posterior nares, accessory salivary glands of base of tongue, parocular, submaxillary, sublingual, and parotid glands, renal pelvis, ureter, and bladder. Rats receiving cod-liver oil showed none of these changes. It is thus seen that the metaplastic changes are as great with a diet deficient in vitamin A alone as in those rats on a diet deficient in vitamins A and D. Since it was conceivable that lack of food alone, in the end stages of the experiments, might induce the changes, rats receiving cod-liver oil, and therefore an adequate amount of vitamin A were starved for a time, but no metaplasia was induced.

Proliferative changes and keratinisation in the trachea of rats have been observed by McCarrison (1930). The proliferative changes appeared to be the result of a chronic pro-

tracted inflammatory reaction, in which varying numbers of mononuclear cells pervaded the subepithelial layer of the mucosa, polynuclear cells being comparatively rare. In some cases the thickening of the mucosa which was thus produced was slight, but in some the membrane was thrown into polypoid or villiform elevations which greatly reduced the lumen of the trachea. These masses closely resembled the hypertrophied, hyperplastic adenoid tissues (adenoids) so frequently found in badly nourished children.

McCarrison also noted changes in the thyroid, consisting of distension of a large proportion of the follicles with colloid material and frequent distortion of their shape, secretory activity being present in parts of the gland not affected by follicular distension. McCarrison states that these observations may be pertinent to other problems. Among these are: the possible relation of faulty food containing an insufficiency of vitamin A to adenoids and the possible relation of insufficiency of vitamin A to iodine metabolism and goitre-formation.

The order of occurrence of metaplasia and hyperplasia in the organs of the vitamin A-deficient rat has been determined by Tyson and Smith (1929).

They have found that this epithelial change attacks the following tissues in this order: "sublingual glands, submaxillary glands, renal pelvis, trachea, and bronchi. Epithelial changes occur in the tongue before xerophthalmia appears, and even in the early stages are associated with infection. The renal pelvis may be involved quite early, and when calculi are present pyonephrosis develops and may be followed by perinephritic abscess. Metaplasia is not common in the trachea and bronchi, and usually atrophy is found. Hyperplasia may be excessive in the tongue and renal pelvis and almost occlude the keratinising process. In the tongue the appearance around an abscess may be hard to distinguish from malignancy. Metaplasia was not observed without infection, but infection was seen in the absence of metaplasia. Some rats which had developed ophthalmia were given cod-liver oil, and the effect of this treatment was observed at different intervals. Epithelial regeneration occurred, varying in extent with the amount of initial destruction due to infection. Kidney infection and epithelial changes appeared to persist and were observed as long as 91 days after the commencement of treatment. It was noted that infection and epithelial changes might persist in the kidney and tongue when the rat was apparently healthy."

(2) **Hypertrophy and Proliferation.**—A striking incidence of epithelial hypertrophy and keratinisation is that described by Fujimaki (1927) at the cardiac end of the stomach of rats fed on a vitamin A-deficient diet. A detailed histological examination was made of pavement epithelium of the fore-stomach, secretory duct of the sublingual salivary gland, pelvis of kidneys, urinary bladder, uterus, vagina, and the epidermis, and of the cylindrical epithelium of the trachea, bronchi, uterus, bile duct, and glandular stomach, in rats fed on a vitamin A-deficient diet for periods varying from 58 to 318 days. Examinations of 49 rats revealed the presence of hyperkeratosis and atypical growth in the fore-stomach and sublingual gland most markedly, and to a lesser extent in the pelvis of the kidney and the bladder. The epithelia of the vagina, uterus, œsophagus, tongue, and skin showed slight changes or were normal. No changes occurred in the cylindrical epithelium. Emphasis is laid on the fact that these changes in the fore-stomach may be carcinomatous since they correspond to the previous findings of Fibiger and Yokogawa, and in 1 case metastatic nodules were found in the lung. These proliferative changes commence with an accelerated state of cornification and epithelial growth, unassociated with any local inflammatory reaction or preliminary ulceration. At the same time there is a round-celled infiltration of the mucosa and submucosa. There was no evidence of parasites in any of the cases.

Further work by Fujimaki and his co-workers (1928) has shown that the atypical epithelial proliferation is slight or absent when the diet is deficient in fat as well as vitamin A. When heated olive oil is added to the diet marked or moderate hyperkeratosis and papil-

lomatous or carcinomatous growths may appear in the fore-stomachs of albino rats. These epithelial changes were produced in a moderate degree by feeding rats on an entirely fatty diet (butter only, butter fat only, or butter fat, 85 per cent., and cod-liver oil, 15 per cent.). Most of the animals fed upon the butter and cod-liver oil diet showed marked degenerative changes in the liver. It is interesting to note that some rats receiving the entirely fatty diet developed xerophthalmia macroscopically similar to that seen in A avitaminosis. The results indicate that the epithelial changes in the fore-stomach are related to a disorder of fat metabolism, and further work is in progress relating to this question.

TABLE, SHOWING SUMMARY OF CHANGES.

Organs.	Number of Animals Examined.	Grade of Changes (Hyperkeratosis and Atypical Epithelial Growth).		
		Marked.	Moderate.	Slight or Normal.
Fore-stomach	49	5 (10%) carcinoma	12 (24%)	32 (66%)
Pelvis of kidneys	39	2 (5%)	2 (5%)	35 (90%)
Bladder	30	3 (10%)	4 (13%)	23 (77%)
Secretory duct of sublingual salivary gland	29	7 (24%)	9 (31%)	13 (35%)

(3) **Spontaneous Tumour Formation.**—The occurrence of spontaneous tumours in rats fed on a vitamin A-deficient diet is described by Erdmann and Haagen (1927, 1928), who believe them to be adeno-carcinomata arising from the mammary gland, though transplantation of portions of the tumour gave negative results. The rats were fed alternately on an A-deficient diet for 3 weeks and then a normal mixed diet for 1 week. Three of 40 surviving rats developed tumours within 7 months. Many of the rats died early from infective processes and 1 developed a papilloma of the fore-stomach, whilst the control rats showed no tumour formation. The histological nature of the tumours is described by Busch (1928): “Mammary gland alveoli, many of irregular shape, were seen to be arranged in lobules, the separating connective tissue being infiltrated with numerous polymorphs, small round cells, and some eosinophiles.” The condition was similar to the functional hyperplasia often found in pregnant rats, but Busch states that the appearances suggest malignancy. He considers that owing to some unknown alteration in local cell metabolism apparently occurring in only a small percentage of animals, the cells respond by abnormal growth to an endogenous irritant produced under these dietary conditions.

(f) **INTESTINAL LESIONS.**—Cramer (1923) has described definite lesions in the lower end of the small intestine of rats on a vitamin A-deficiency diet. These changes consist of profound atrophy of the villi and necrosis of the upper parts of many of the villi. (See Plate I.)

The intestinal bacteria also appear to be present in larger numbers than normally and can often be seen adherent to the villi, especially the necrotic tips. In the cæcum the bacteria often invade the mucous glands and are found in dense masses filling the lumen of the gland completely. In normal rats such a condition may occur exceptionally, in a few glands, but in a rat suffering from A deficiency this is the rule, and affects a great many of the glands of the cæcum. In addition to these gross changes an abundance of protozoa, mainly *Giardia intestinalis* (*Lambia intestinalis*), are found in the lumen of the intestine of rats in advanced vitamin A deficiency. These are probably normally present in the intestine in small numbers, but the absence of vitamin A enables them to proliferate rapidly, and to penetrate down between the villi, where normally they are never present.

The Figure or Plate I shows a gland which is actually distended by a mass of bacteria

in addition to a few round bodies, probably encysted protozoa. The neighbouring gland, of which only the opening is shown in the drawing, is also invaded by bacteria but not distended. Cestodes, also, are present much more frequently in rats kept on a vitamin-free or vitamin-restricted diet than in rats on a vitamin-rich diet.

Gross (1920) and Connell (1913) failed to confirm the occurrence of extreme atrophy of the intestine observed by Cramer, but made an interesting observation on the effect of vitamin A-deficient diet on the motor function of the intestinal tract. By incorporating charcoal in the diet it was possible to estimate the time taken by the food to pass through the entire tract. In vitamin A deficiency the time taken for the charcoal to disappear was considerably shortened. The faeces were grey and somewhat lacking in bile pigments, as in vitamin B deficiency, but in spite of the alteration in the secretory functions of the alimentary tract thus indicated, there was no initial stasis but rather a peristalsis.

(g) **DIMINUTION OF BLOOD PLATELETS.**—According to Cramer, Drew, and Mottram (1922), a diminution in the number of platelets can be recorded in vitamin A-deficient rats independent of their growth propensities, and this change becomes markedly manifest before the infective conditions make their appearance. The average of the platelet counts obtained by these workers in normal rats is 851,000 per c.mm., whilst the average of their counts in vitamin A-deficient rats is 378,000, the lowest platelet recorded by them in this latter category of rats being 154,000 per c.mm.

In a later paper (1923) Cramer and Drew suggest that platelets are formed by the endothelial cells of lymphatics and blood vessels, a view which agrees with the findings of Bedson (1922) that an antiplatelet serum, which is highly specific for platelets, has also a toxic effect on the endothelium of the blood vessels. They have proved that conditions which stimulate the production of platelets (such as light and vitamin A) will delay the development of bacterial infection, and consider this fact a further proof of the view that platelets represent a mechanism against bacterial infection.

Bedson and Zilva (1923), however, have been unable to find in confirmatory experiments sufficient evidence to justify the statement that "thrombopenia" is a simple lesion of vitamin A deficiency. They found, nevertheless, that young growing rats fed on a vitamin A-deficient diet show a lower platelet count than do normal rats of the same age, the greatest diminution in the platelet count observed by them being 244,000, representing a 21·3 per cent. reduction.

(h) **CHANGES IN BONE TISSUE.**—The changes in bone tissue due to deprivation of vitamin A have been investigated by Frances M. Tozer, who has attempted at the same time to differentiate them from those which occur in vitamin C deficiency. She found that the bones of animals whose diet contained no vitamin A but a sufficiency of vitamin C were fragile and exhibited minute ridges at the rib junction. The teeth also were brittle and worn down. Histologically, the early changes (14 days) in the costo-chondral junction were indistinguishable from those occurring in the ribs of scurvy animals at a slightly later period. Later changes (21 days) showed a condition similar to that met with in cases of subacute or partially protected scurvy—a considerable reduction in length both of the trabeculae and the rows of cartilage cells. These changes were progressive, and by 30 to 40 days the rows of cartilage cells and trabeculae had almost disappeared. The cartilage often ended in a thin, ossified band, which was apparently laid down in an attempt to buttress up a weak joint. After 50 days, owing to the increased fragility of the bones, fractures of some of the ribs occurred and the sections showed proliferation of cartilage cells.

When the animals were simultaneously deprived of vitamin A and C it was not found possible, histologically, to distinguish with certainty mild cases of deprivation of vitamin A, nor to state, from observation of the bone tissue alone, whether an animal has suffered slight simultaneous deficiency of both accessory factors. Histological appearances at the costo-

chondral junction in animals which had survived deprivation of vitamin A for over 60 days bearing some resemblance to rickets were also detected. The cartilage cells were found to have lost their orderly progression, the blood cells entering irregularly. A special function has been allocated to vitamin A in connection with the growth of bone by Harris (1926). He analyses skeletal-growth processes in these separate areas: that of cartilage proliferation for which he holds the water-soluble vitamins responsible; that of degeneration of cartilage, related to deficiency of vitamin D; and that of ossification proper, the specific function of vitamin A, which he considers the factor *par excellence* of tissue differentiation.

(i) **CHANGES IN BONE MARROW.**—In animals fed on diets deficient in vitamin A it is rare to find lesions in the leucoblastic bone marrow, but in a very few adult animals on prolonged vitamin A deficiency an aplastic condition of the marrow has been reported by Findlay and Mackenzie (1922).

Frances M. Tozer, in the experiments reported above, found, in cases of 21 days' deprivation of vitamin A, some slight atrophy, but no hæmorrhage, of the bone marrow. Later, progressive atrophy occurred, and in extreme cases little but the delicate reticulum of the connective tissue groundwork and the blood vessels remained.

After 50 days sections of some of the fragile ribs showed development of connective tissue in the marrow cavity, which, being present in the same position in deprivation of vitamin A as in cases of severe scurvy, tends to prove that its occurrence is not in itself a scurvy symptom. The fibrous tissue is presumably developed in an attempt to repair fracture of the fragile bone rather than by symptoms of either vitamin A or C deficiency.

(j) **CHANGES IN THE BLOOD**—(1) **White Cells.**—There is no reduction of the number of leucocytes in the blood stream in vitamin A deficiency; in fact, according to Findlay and McLean (1925), it is not uncommon to meet with a polymorphonuclear leucocytosis after the onset of keratomalacia or broncho-pneumonia.

(2) **Red Cells.**—Koessler, Maurer, and Loughlin (1926) have reported a well-marked decrease in both the number of red cells and the amount of hæmoglobin in the blood of rats fed on a diet deficient in vitamin A.

Falconer (1926), investigating the changes in the relative number of platelets, red cells, and white cells in blood of rats fed on a vitamin A-deficient diet finds them not constant enough to constitute a specific lesion in vitamin A deficiency.

On the addition of small quantities of vitamin A there are signs of increased bone-marrow activity, but there is a long latent period before the hæmoglobin and erythrocytes in the circulation are obviously increased. If vitamin A is now removed a second time from the diet and then given again, a blood picture closely resembling that of pernicious anæmia is produced; vitamin A deficiency has produced a severe anæmia and the subsequent addition of the vitamin has caused blood regeneration.

(3) **Immunological Properties, etc.**—The immunological properties of the blood are unchanged. The bactericidal power is diminished, but Findlay points out that, since a reduction of this factor occurs in all acute infections, the lack of vitamin A in the food does not directly cause the fall. The phagocytic activity, also, according to Findlay and McLean (1925), and Smith and Wason (1923), is perceptibly reduced at the moment when keratomalacia appears.

(k) **DISTURBANCE OF REPRODUCTIVE FUNCTION.**—Evans and Bishop in 1922 reported a change in ovarian function in vitamin A deficiency, an arrest of the œstrous cycle. This change is characterised by the appearance of keratinised cells and the disappearance of leucocytes. Drummond considers the presence of cornified epithelial cells, signified also by Macey, Outhouse, Long and Graham (1927), as the most trustworthy and characteristic sign of vitamin A deficiency, appearing before other better known signs, such as cessation of growth and xerophthalmia.

Parkes and Drummond (1926) report that rats in which growth has ceased owing to a vitamin A deficiency are sterile. Histological examination of the gonads and secondary organs usually revealed no anatomical reason for sterility. Gametogenesis was found in these stunted animals, and the accessory sex organs appeared normal. It was concluded, therefore, that the sterility was due primarily to physiological debility and disinclination to copulate. This theory proved true in a further group of deficiency rats which were reciprocally mated with each other and with normals, daily examinations being made for evidence of copulation. None of the females copulated. One only slightly deficient male copulated with a normal female. At the same time vaginal smears showed that the cyclic activity associated with the oestrous cycle occurred in the deficient female. When normal weight was attained after recovery on natural diets, breeding took place freely, and fertility and sex-ratio showed no marked abnormality.

Evans (1928) considers that the effect of the vitamin A deficiency is an injury to the female reproductive system quite different from the resorption phenomenon of vitamin E deficiency, causing frequent failure of fertilisation and implantation. He states that the cornified cell smear, typical of vitamin A deficiency, exists throughout pregnancy whenever it occurs.

Coward (1929) and Coward, Morgan, and Dyer (1930), however, state that the continued occurrence of cornified cells in the vaginal smear of a rat cannot be relied upon as an indication of vitamin A deficiency, for (a) the vagina may not open until some time after the usual signs of vitamin A deficiency have shown themselves, and (b) when the vagina opens during the resumption of growth following the administration of vitamin A., leucocytes only may appear in the vaginal smear for many days before normal cycles are established.

Sure (1928) reports the production of sterility characterised by resorption of the foetus during gestation and associated with vitamin A deficiency. The diet consisted of skim milk powder and is stated to contain an abundance of vitamin E.

(l) **UROLITHIASIS.**—In 1917 Osborne and Mendel drew attention to the frequency of calculi in the urinary tract of rats on a diet deficient in vitamin A. In 857 necropsies they found calculi eighty-one times. "In every instance," they wrote, "where calculi developed, the animals were without an adequate source of the fat-soluble vitamin for some time." They also noted that in their earlier work they had often used diets which were deficient in fat-soluble vitamin, whereas later this deficiency had not been permitted.

McCollum and Simmonds, however, failed to confirm these results, and McCollum stated in 1924: "Osborne and Mendel observed calculi of calcium phosphate in the urinary tract in 91 (read 81) animals among 857 necropsies; 43 per cent. of these had not had a satisfactory supply of vitamin A. In McCollum and Simmonds's experience calculi have occurred so frequently in animals whose diets contained an abundance of this factor, but were faulty in other respects, that it would seem to be the results of general debility rather than lowered vitality brought about by specific cause."

Confirmation of Osborne and Mendel's work, however, has been received from several quarters.

Fujimaki (1926) found that a deficiency in vitamin A regularly produced calculi not only in the bladder but also in the kidney and gall-bladder. The first did not develop consistently until the rats had been on the diet for 12 weeks or more; renal stones took a longer time, and gall-stones longer still. The rats were kept alive for these long periods by being occasionally given a normal diet for a short time; the principal difference lay in an addition of cod-liver oil. Fujimaki pointed out that the change from the deficient diet to the one containing cod-liver oil was accompanied by a change in the reaction of the urine from alkaline to acid. He was actually able to demonstrate that stones, detected by X-ray, disappeared after the rat had received a course of cod-liver oil.

McCarrison (1927) has obtained very similar results. The diet he used was the following : Scotch oatmeal, 636 grms. ; linseed meal, 240 grms. ; tinned cornflour, 300 grms. ; NaCl, 12 grms. ; $\text{Ca}_3(\text{PO}_4)_2$, 12 grms. ; distilled H_2O , 450 c.c. The protein content of this diet is low, and being of vegetable origin the protein is of low biological value. The diet is rich in phosphates, lime, and magnesia, and relatively, though not entirely, deficient in vitamin A. If fed for longer than 56 days on this diet there was a tendency for stones to form in the bladder. Of 72 animals, 29 per cent. were found to have stones, often associated with cystitis.

In a further series of experiments the oatmeal in the above diet was replaced by whole wheat flour (*atta*), and the revised diet proved more calculus-producing than the original one. In some cases dilatation of one or both ureters was found. Hydronephrosis, pyonephrosis, and stone in the kidney were occasionally found, and though cystitis was usually found associated with stone in the bladder this was not invariable. The main faults of the diet used are its high cereal content, its vitamin A deficiency, and its excess of phosphates. In these respects it approaches in quality that used by the inhabitants of stone areas in Northern India.

Whole milk in amounts of two-thirds of an ounce daily was added to the daily ration of half of a series of rats on the oatmeal diet. Fifty per cent. of the rats fed without milk were found to have formed stones or gravel in the bladder (all died within periods from 66 to 150 days). The animals receiving milk grew well as a rule. When killed (167th day), neither stone in the bladder nor any abnormality of the urinary tract was found.

Experiments were carried out to determine whether linseed meal or oil possessed some toxic quality which produced the calculus-forming nature of the diet. No significant differences could be found between diets containing small or large amounts of linseed or when the linseed oil was replaced by sesame oil. It has since been found that stone in the bladder may also occur in rats surviving for long enough periods (120 to 130 days) on an exclusive diet of white flour. It is therefore concluded that stone in the bladder in rats is due to an insufficiency, in the diets used, of a substance or substances (probably vitamin A) which whole milk of good quality provides in abundance. This substance is not present in sufficient quantity in certain of the vegetable oils (linseed and sesame) in common use in India.

Van Leersum (1928), examining a number of rats in the Netherlands Institute of Nutrition in Amsterdam, came to the same conclusion. Out of 886 rats, 241 receiving adequate amounts of vitamin A showed no signs of lithiasis, whilst of the other 645 rats, all receiving A-deficient and in some cases A- and D-deficient diets, 197 showed evidence of urinary salt deposit. The bladders which were apparently without calculi were examined histologically, and in this way concretions invisible to the naked eye were occasionally revealed. The calculi mainly consisted of calcium phosphate or a mixture of calcium phosphate and calcium oxalate. A number of rats had hæmaturia, but in many cases hæmaturia occurred without lithiasis, and *vice versa*. Obvious cystitis was rare, the urine was usually acid, and in most cases of lithiasis the bladder mucous membrane was intact. Lime deposits were constant throughout the kidney tubules (87 per cent. of the rats on vitamin A-deficient diets, compared with a frequency of bladder calculi of 35 per cent.), the glomeruli being unaffected, whereas in adequately fed rats lime deposits of any size were exceptional. These observations were controlled by removing one kidney in a series of rats and then feeding on an A-deficient diet ; lime deposits appeared quickly in the kidney tubules, in one case in 13 days. It is suggested that keratinisation of the renal epithelium cells favours the deposition of lime, which deposition, once liberated, grows to larger concretions in the bladder.

Perlmann and Weber (1928) have also found stones in the bladder in 3 out of 15 rats who survived for 5 months on a vitamin A-free diet. (Vitamin D was apparently also absent, the diet consisting of barley, refined rice, yeast, poor margarine, and salts.) No stones or pathological changes were found in the kidneys or ureters, but the base of the bladder was

injected, and the urine was cloudy in some cases. As a result of their later experiments (1930), these workers have concluded that vitamin A deficiency is not the only factor, though an important one, in stone formation. They believe also that the nature of the diet influences very decidedly the chemical nature of the stone. In Europeans, urate stones predominate, whilst in the Japanese, oxalate stones are commoner.

The type of stone formed is, according to de Langen (1929), very similar to the inflammatory stone of human pathology. De Langen's findings agree with those of Fujimaki, especially with regard to the occurrence of gall-stones, as well as bladder and kidney stones, earlier in the experiment when the diet was deficient also in calcium and protein. The stones were clearly inflammation stones, always contained some pus cells and necrotic tissue or coagulated blood. The urinary stones consisted of phosphates, calcium, and magnesium, while the gall-stones were composed chiefly of bilirubin pigment, and contained little cholesterol.

Although stone formation is prevalent in Java, de Langen states that the diet of the population shows no deficiency in vitamin A. He explains the apparent discrepancy by the facts that a state of low nutrition exists generally among native children, on account of limitations of diet for intestinal disturbances, which are common. He thinks that the intestinal disorders affect the absorption of the little vitamin A in the diet, and also interfere with the water metabolism, so that the urine becomes concentrated and therefore liable to primary crystallisation. Data from the Netherlands East Indies show that 67 per cent. of bladder stones occur in the age period 1 to 15 years, but in most cases symptoms are referred back to early childhood, and probably 80 to 90 per cent. of cases begin at this time.

(*m*) **METABOLIC CHANGES.**—Synthesis of purin bodies is diminished in vitamin A deficiency according to Morgan and Osburn (1925). These authors state that in the normal animal the excretion of allantoin diminishes with a gain in weight, and increases as the weight falls, while an exactly opposite condition of affairs holds good in the case of the vitamin A-deficient animal.

Emmett and Peacock (1925) describe a disturbance of uric acid metabolism, shown by bands of white incrustation of the liver parenchyma. Hypercholesterolaemia, induced in rabbits by injections of cholesterol, is stated by Kimura (1928) to return to normal slowly when vitamin A in the form of biosterin was administered than in control animals.

(*n*) **LIVER FUNCTION.**—The excretory function of the liver in vitamin A deficiency has been examined by Saiki (1929) by injecting an organic dye into the blood stream and determining the amount of the dye in the bile after certain time periods. In the control group of rats, azorubin S appeared in the bile $1\frac{1}{2}$ to $3\frac{1}{2}$ minutes after injection, and about 60 per cent. was secreted in $1\frac{1}{2}$ to 2 hours, the first 30 minutes being the period of most active excretion. In the rats on the A-deficient diet the dye was excreted much less rapidly, although the time of appearance in the bile was not much different from the control group.

(*o*) **SUSCEPTIBILITY TO POISONS.**—Gross (1924) states that rats fed on a vitamin A-deficiency diet are susceptible to arsenical poisoning.

(*p*) **LACK OF RESISTANCE TO INFECTION.**—Experiment suggests that vitamin A is more directly related to resistance to infection than any other food factor. The part played by it as an anti-infective agent has been investigated both from the specific and the general point of view.

(*q*) **SUSCEPTIBILITY TO LUNG INFECTIONS.**—Several workers, including Drummond, Cramer, and Kingsbury (1924), and Steenbock (1924) have commented on the susceptibility to lung infections of animals on vitamin A-deficient diets.

Drummond (1919) has described a lowering of resistance of adult rats to bacterial infection, manifesting itself sometimes in the form of an inflammatory condition of the lungs, when they were fed on diets deficient in vitamin A. Cramer and Kingsbury have also called

attention to the mortality of rats from broncho-pneumonia when these animals are kept sufficiently long on diets free from vitamin A, and H. C. Sherman and F. L. McLeod were extremely struck with the liability to broncho-pneumonia, induced in rats by a deficiency of vitamin A.

In the case of dogs, E. Mellanby (1926) drew attention to the fact that a tendency to broncho-pneumonia in animals living on diets deficient in fat-soluble vitamins was independent of the condition of the bones, which is itself an indication of the vitamin D intake. Atelectasis and broncho-pneumonia were found when olive oil replaced butter or cod-liver oil, and the results suggested that protection against infection of the respiratory tract was conferred by vitamin A and not by vitamin D. In some cases, at least, the broncho-pneumonia developed in animals which not only had a diet deficient in vitamin A, but also had been taken out of their indoor kennels into the open air, where it was usually cold and windy and often wet, in order that their running powers should be determined. The opportunity, in fact, was presented to them of catching a "chill." The dogs on the diets containing vitamin A were also placed under the same conditions, but their resistance was apparently sufficient to make the low temperature of the external conditions of no account.

Attempts to trace catarrhal infections, particularly of the respiratory tract, in children, to a mild insufficiency of vitamins, have been made by Mellanby (1926), basing his observations on the above experiments, and on those of Bloch of Copenhagen, and Blegvad of Denmark.

Block (1921) described an outbreak of glanular conjunctivitis in an institution where the children received no milk, fat, or butter, as an epidemic of xerophthalmia, and only mentioned incidentally that the children suffered from broncho-pneumonia, pyuria, etc. Subsequently an inquiry into the occurrence of xerophthalmia throughout Denmark was reported on by Blegvad (1923), who showed that when this condition terminated fatally the cause of death was in the great majority of cases broncho-pneumonia, and in the remainder enteritis.

I. G. Macy and co-workers (1929) have noted otitis media, mastoid disease, and sublingual abscesses, as well as the usual lung disease in fat-soluble vitamin deficiency; while Funk (1927) suggests that the severe influenza epidemics during the war were probably the result of defective nutrition—a suggestion supported by the disappearance of an epidemic of pneumonia in the Sudan, coincident with the antiscorbutic treatment of numerous cases of scurvy which arose at the same time.

(1) In the 1927 Report of the Committee on Nutritional Problems, presented to the American Public Health Association, it is stated that in cases of respiratory diseases in experimental animals, whose food is relatively poor in vitamin A, there is an actual diminution of the vitamin A content of the lung tissue.

(2) **Tuberculosis.**—Smith and Hendrick (1926) have observed that the tubercle-infected rat deteriorates more rapidly than the non-infected control when maintained on a diet of low biological value and low in fat-soluble A. Liberal allowance of vitamin A appears to afford protection against the early deterioration, indicating either a lowered resistance in the rat to tubercle infection or an increased demand for vitamin A.

(3) **Common Pyogenic and Urinary Tract Infections.**—Green and Mellanby (1928) have made an extensive investigation of the infective lesions that develop in animals kept on a prolonged vitamin A-deficient diet.

In the 93 A-deficient animals examined, the most characteristic condition was the lack of adipose tissue and the general visceral atrophy, but almost as prominent was the evidence of infection in some site, only 2 animals of the 93 escaping entirely. The production of vitamin D by irradiation of ergosterol, as described by Rosenheim and Webster (1927), made

it possible to give diets deficient only in vitamin A, though the addition of vitamin D made little difference to the results. In fact, it often appeared that the addition of vitamin D in the absence of vitamin A hastened the onset of the infective condition. The reason for this is probably that the presence of vitamin D stimulated the rate of growth, and thereby made a greater call on the vitamin A stores of the body, thus hastening the diminution of resistance.

The following account of their results was given by Green and Mellanby :

“ Abscess at the base of the tongue, arising in the accessory salivary glands, was found in 72 per cent. of the animals. In animals surviving for longer periods this figure rose to 90 per cent. These abscesses varied much in size, some being small caseous foci in the centre of the gland, while others involved almost the whole tongue, infiltrating it almost to the tip. Perforation of a tongue abscess was not uncommon. In one case a sinus communicated with the skin of the anterior surface of the neck, and in another perforation into a large artery resulted in death from hæmorrhage. Associated with the tongue abscesses, suppuration occasionally occurred in the submaxillary glands and in the lymphatic glands of the neck. In some cases a chain of suppurating lymphatic glands was seen in the neck, the glands being usually quite discrete. The thyroids were involved in the suppuration in one animal.

“ Infection of the lungs was noticeably rare, only 9 per cent. showing any definite signs, which were chiefly of a septic broncho-pneumonia. These cases all occurred during the winter months, though the temperature of the rat-room was kept constant.

“ Infection of the alimentary tract was quite common, though in most cases it appeared as a terminal event, there being no clinical evidence of its occurrence. An acute inflammation of the small or large intestine occurred in 21 per cent. of the rats. Many of these cases arose during an epidemic of enteritis in the rat-room when, as previously mentioned, the vitamin A-deficient animals proved to be very susceptible to the infection. In quite a number of animals an acute enteritis involving the duodenum and jejunum appeared to be the immediate cause of death. The intestine was grossly injected and filled with a copious hæmorrhagic exudate. Usually there were signs of recent hæmorrhage into the pyloric portion of the stomach.

“ Lesions of the urinary tract often dominated the scene in the abdomen. The occurrence of calculi—usually in the bladder, rarely in the kidney—was fully confirmed, but infection occurred independently of their presence or absence ; 44 per cent. showed evidence of infection either in the kidney or bladder, but the incidence was considerably higher in those rats surviving longer periods. The infection took the form of a pyelonephritis or cystitis, and sometimes pus was found in the kidney substance. The infection was usually associated with such conditions as hydronephrosis, extremely dilated ureters, and a distended bladder. Multiple small white necrotic foci in the cortex of the kidney were often found, but their exact origin has not yet been determined. Similar foci have also been observed in the spleen on two occasions.

“ The prostate glands and seminal vesicles were frequent sites of suppuration, whilst occasionally the epididymis was acutely inflamed or suppurating. A purulent exudate was seen in the Fallopian tubes on one occasion.

“ Pus was found in the nasal sinuses or in the middle ear on eighteen occasions—an incidence of 20 per cent. In one isolated case a small but definite abscess was found projecting from the wall of the left ventricle of the heart.

“ The control animals received the basal diet *ad lib.*, plus some source of vitamin D, with the addition of a source of vitamin A either in the form of dried cabbage or cod-liver oil or butter. Fifty rats fed under these conditions over periods as long as, or longer than, those which the deficient rats survived have been killed and examined. In no single case was

evidence of gross infection found corresponding with that found in the vitamin A-deficient rats. The only pathological findings were three cysts of the liver, of parasitic origin. Otherwise all the tissues appeared perfectly healthy. The evidence for infection was in every case macroscopic, being based either upon the presence of pus or of an acute inflammatory process."

On the basis of these facts, Mellanby suggests that vitamin A plays a significant part in raising the bodily resistance to infection, possibly owing to the favourable medium which obstruction by desquamated keratinised cells provides for bacterial growth.

Sherman and Burtis (1928) have shown that the vitamin A requirement of the body, particularly with regard to susceptibility to infection, increases both with the size of the body and the rate of growth—a fact which indicates the necessity for a liberal allowance of this vitamin in growing children.

Two groups of rats were fed under identical conditions on a vitamin A-free diet. One group (A, 38 rats) had been reared up to 4 weeks of age on a diet containing $\frac{1}{8}$ dried whole milk and $\frac{5}{8}$ ground whole wheat; the other group (B, 37 rats) had been reared to the same age on the same food with a larger proportion of whole milk ($\frac{1}{3}$ of dried weight). All the rats then received an A-deficient diet for 4 weeks and then were given the same limited allowance of vitamin A for 8 weeks. At the end of this period, infection was established in 75 per cent. of group A and in only 25 per cent. of group B. It is considered that this represents the incidence of infection to be expected in children around 10 to 12 years resulting from differences in the way they were fed up to 3 years. The superior diet was also richer in vitamin B₂, in Ca, and certain amino acids.

Similar conclusions have been reached by Frontali (1926) with regard to infection of the urinary tract. White rats on a diet deficient in vitamin A showed, besides xerophthalmia and, in many cases, multiple subcutaneous abscesses, infection of the urinary tract. The lesions were those of perivascular hæmorrhage in the cortex and medulla, and pus in the pelvis of the kidney, urethritis, and cystitis with submucous infiltration.

(4) **Puerperal Septicæmia.**—Mellanby and Green (1929) have recently suggested a specific action of vitamin A with regard to puerperal septicæmia. On the hypothesis that the storage of vitamin A in the body is called upon during pregnancy, they attempted prophylactic experiments by giving a diet rich in vitamin A in the antenatal period and comparing the incidence of puerperal sepsis with a number of controls. They state that the results, though not conclusive, are in favour of the hypothesis.

They also investigated the curative properties of concentrated preparations of vitamin A in 5 cases of puerperal septicæmia from whose blood hæmolytic streptococci were grown.

The two preparations used were respectively about 10 times and 150 times as rich in vitamin A as cod-liver oil. The following table represents their results :

					Prior to Vitamin A Treatment.		Vitamin A Treatment.
					1927.	1928.	1929 (3 months).
Number of cases	8	16	5
Number of recoveries	2	0	5

Although the results are too few to warrant the deduction that vitamin A is specific for septicæmia, they tend to confirm Mellanby's previous observations as to its efficacy as an anti-infective agent.

(5) **Streptococcal Infections.**—It would be, in fact, necessary to prove that vitamin A had a definite effect on antitoxic immunity in order to claim specificity for it as a prophylactic and anti-infective agent. This aspect of the question has been recently investigated by Burton and Balmain (1930) with results which do not warrant the conclusion that vitamin A is a valuable prophylactic agent against streptococcal infection. They carried out their

experiments on the antitoxic immunity as demonstrated by the Dick tests in (a) a group of pregnant women attending an antenatal clinic, who presumably had no stimulus provided by a specific antigen. Although a certain number of the cases which were Dick-positive at the initial test became Dick-negative later, these cases had not a large reaction, while some returned to their original Dick-positive condition, and a large percentage remained quite unchanged. Burton and Balmain concluded that the fact that the administration of vitamin A in 36 cases had not the slightest effect was sufficient demonstration that this vitamin, like other non-specific substances, cannot cause an appearance *de novo* of antitoxin; (b) patients suffering from scarlet fever who had of course a specific stimulus provided by a streptococcal antigen. Whereas 34.4 per cent. of the cases to whom vitamin A was given were Dick-positive on discharge from hospital, 39.3 per cent. of cases in the control series were positive. The difference of 5 per cent. cannot be held to show that any material effect was produced by the administration of vitamin A.

Burton and Balmain believe that the small difference was due to such factors as the infecting type of streptococcus and the individual power of developing immunity. They conclude that there was no difference in the production of antitoxin in the cases which received vitamin A and those to whom it was not administered. On the whole their results seem to lead to the conclusion that there is no evidence at present that the administration of vitamin A in pregnancy has any effect in increasing the resistance of the body to streptococcal infection. Whether or not it has any effect in raising the general resistance is unknown and impossible to determine.

Cramer (1930) considers that the anti-infective action which vitamin A does possess is excited by virtue of its function in maintaining the physiological defences of the mucous membranes. As already stated, he has shown (1923) that in vitamin A deficiency there occurs, particularly in the lungs (McCarrison, 1922) and intestine, an atrophy of the mucous-secreting cells, allowing penetration of bacteria. Cramer and Kingsbury (1924) have also pointed out that animals kept on a vitamin A-deficient diet do not differ in their immunological reactions from normal animals nor are they more susceptible to infection by virulent or avirulent pathogenic organisms, when these are introduced experimentally by subcutaneous injection. From these observations Cramer concludes that adequate supply of vitamin A is a powerful physiological prophylactic against infections entering by the mucous membranes, but there is no evidence that vitamin A can cure infections once the barrier of the mucous membranes has been passed or that it can prevent or cure those infections which enter by the blood stream or by the subcutaneous tissues, as they do, for instance, in puerperal septicæmia.

ORGANISMS IN INFECTIONS FOUND IN VITAMIN A DEFICIENCY.

(1) **In the Common Pyogenic Infections.**—Most workers, including Mellanby, have found no specific organisms in the infected tissues and in pus. In Mellanby's experiments Gram-positive diplococci were often found, but these were usually associated with streptococci, staphylococci, and Gram-positive and Gram-negative bacilli. Occasionally the pus from infected glands of the neck showed a few chains of streptococci and no other organisms.

(2) **In Respiratory Tract Infections.**—An encapsulated bacillus of the mucosus group has been isolated by Bradford (1928) from about half the suppurative terminal lesions in white rats on a vitamin A-deficient diet. Bradford considers that this bacillus is probably a secondary invader of mucosa of the respiratory tract, made suitable for its invasion by dietary deficiency. Bacterial invasion of the upper respiratory tract and middle ear in rats on a vitamin A-deficient diet was found by Turner, Anderson, and Loew (1930) to consist chiefly of *Staphylococcus aureus*, *B. coli*, *M. catarrhalis*, and Gram-negative cocci. These

latter (called chromogen 6) were found most frequently in animals which showed the most severe symptoms of vitamin A deficiency. The above workers conclude that these organisms gain a pathogenic hold during the depressed state which occurs in A-avitaminosis.

A study has been made by Shurly and Turner (1930) of the pathogenicity of the organisms isolated from the upper respiratory tract in rats suffering from vitamin A deficiency. Following up the observations of Clark and Murphy (1921) that the bacterial flora of the upper respiratory tract frequently consist of Gram-negative cocci, and of other workers, including Ghon and Pfeiffer (1902) and Elser and Huntoon (1909), that these organisms are relatively non-pathogenic, they investigated the virulence of these organisms in vitamin A deficiency. The methods of testing were: (1) intravenous injections of 4 c.c. of a 48-hour dextrose broth culture into the marginal vein of a rabbit; (2) inoculation of rabbits with standardised vaccines. Their results lead them to conclude that vitamin A deprivation leads to marked augmentation of the virulence of ordinarily harmless types of bacteria. They did not, however, test the pathogenicity of the organisms by their hæmolytic properties, nor take into account such factors as decreased resistance as shown by a differential leucocyte count, endocrine disturbance, etc.

(3) **In Urinary Tract Infections.**—In Frontali's experiments, *B. coli* and *Staphylococcus aureus* were isolated from the pus. The intestinal bacterial flora of rats have been shown by Creekmur (1922) to undergo a change when the rats are placed on a vitamin A-deficient diet. On the deficient diet the total number of viable bacteria is greatly decreased and streptococci disappear almost entirely. The proportions of viable organisms fermenting glucose, lactose, and saccharose, and forming hydrogen sulphide, remain constant.

An investigation was undertaken by Verder (1929) to ascertain whether symptoms of "food poisoning" might follow the feeding of *Salmonella enteritidis* to rats on diets deficient in vitamin A. *S. enteritidis* was isolated from the spleen, ileum, and large intestine of both the controls and the experimental animals.

Other organisms, however (coli-like and proteus-like bacteria, staphylococci, and streptococci), were found in various organs of the vitamin A-deficient animals, while those of the controls contained no bacteria of types other than *S. enteritidis*.

(r) **"BLACK TONGUE" IN DOGS.**—A condition is described by Underhill and co-workers (1928), similar to that which Goldberger (1928) considers to represent pellagra. Underhill's syndrome, however, is curable by substances which include butter, pig's liver, eggs, carrots, and recrystallised carotin. In view of the recent evidence by Moore (1928, 1929), Euler and co-workers (1928, 1929), and McLean and co-workers, that carotin possesses an intense physiological activity comparable to that of vitamin A, it is suggested that this form of "black tongue" may be a form of vitamin A deficiency.

(s) **PERNICIOUS ANÆMIA.**—Mellanby (1930) suggests that the close relationship of the cord degeneration found in pernicious anæmia and the changes produced by cereals in dogs in the absence of vitamin A indicates similarity in ætiology. He points out also that in his experience the blood changes in pernicious anæmia cannot be cured by the fat-soluble portion of liver, while, according to Ungley and Suzman (1929), the nerve symptoms can be greatly improved by feeding whole liver. Mellanby has found that the nerve degeneration of ergotism and that produced by wheat germ or embryo can be prevented or, if developed, the condition greatly improved by the fat-soluble portion of liver. He suggests the possibility that both the blood changes and the cord degeneration in pernicious anæmia are due to failure on the part of the liver, the blood condition being due to the exhaustion of some specific water-soluble substance and the cord changes to a deficiency of a specific fat-soluble substance, probably vitamin A.

XXIV. STORAGE OF VITAMIN A.

The liver appears to be an important centre of vitamin A storage, varying in content with the ration fed. The possibility that more vitamin A is stored by animals suffering from rickets than by normal animals is indicated by the experiments of Gibbons and Barney (1930). Rats fed on the livers of rachitic hogs showed a better growth than those fed on the livers of normal hogs, showing that rachitic liver is a better source of vitamin A than normal liver. Gibbons and Barney suggest as a reason the fact that rachitic animals grow more slowly than normal animals and are therefore able to store and conserve more of the vitamin A supplied in their food.

The experiments of Steenbock, Sell, and Nelson (1923), and of Wilson (1927), leave no doubt that the rat can store the fat-soluble vitamin in large amounts in its tissues for future use. Wilson found a fatty extract from the human liver to contain a substance giving the same colour reaction as vitamin A in an amount which may be equal to twenty-five times the amount found in good cod-liver oil.

Sherman and Cammack (1926) found that young rats, which had had cod-liver oil added to quite a good diet for 2 months before being fed on the deficient diet, lived very much longer than those which did not receive the addition of oil. Rats which had received an addition of 2 per cent. of cod-liver oil survived the subsequent deprivation of vitamin A longer than those that received 1 per cent., and those that had received the oil for 2 months lived longer than those that had received it for 1 month. Even one day's feeding with cod-liver oil might, under certain circumstances, increase the survival period by 50 per cent. It appears that both when the level of intake of vitamin A is moderate and when it is very liberal, young animals continue to increase their bodily store throughout the entire period of rapid growth. The livers from 71 cases of various types of disease in man were tested by Laqueur, Wolff, and Dingemanse (1928). Extreme variation in the vitamin A content was found (from 0 to 160 units per gram of liver substance [note, not liver fat]), but the number of each type of case was too small to enable any general conclusions to be drawn.

ABSORPTION OF VITAMIN A.—Subcutaneous injection of butter oil is stated by Koehne and Mendel (1929) to cause some possible utilisation of vitamin A. Intramuscular injection was also found by Mori (1922) to have a favourable effect. Parenteral injection, however, is not a dependable method of introducing vitamin A. Stepp (1925) reported a good effect, and Wollman and Vagliano (1923) also, if the dose were small (0.5 c.c.), but larger doses were toxic.

XXV. PATHOGENESIS OF VITAMIN A DEFICIENCY.

As in the case of all other avitaminoses the cause of decline in health is essentially a failure of the animal, in the absence of vitamin A, to utilise the elements of an otherwise normal diet.

The actual physiological basis of the cessation of growth in vitamin A deficiency is as yet incompletely understood.

Drummond (1919) found no evidence of a disturbed fat metabolism, though Takahashi maintained that there was a direct relation between the amount of vitamin A required and the amount and molecular weight of the fat in the diet.

Randoin and Simonnet (1927) state that the part played by vitamin A in metabolism is in regard to lipoids, either because it represents a constituent element of some of them or because of its great oxidative capacity, as, for example, in the formation of fats from glucides.

Fats disappear little by little in deficient animals at an earlier date than in similar animals on a diet which is merely deficient in total quantity. In this connection it is interesting

to note that Bloch (1924) was able to cure xerophthalmia in a boy who was unable to take fat by the mouth by giving vitamin A-bearing fat parenterally.

Cramer (1923) suggests that the explanation of cessation of growth lies in the lesions of the intestinal mucous membrane—atrophy and necrosis of the villi—which he has found present in rats fed on vitamin A-deficient diet. He points out that such an atrophic condition must interfere with the absorption of food.

In the case of xerophthalmia, the question arises of permeability of epithelium. Randoin and Simonnet suggest that the trouble is due also to the production of a toxic substance with a selective action upon the cornea, just as certain poisons act selectively upon certain types of cellular tissues, *e.g.* chloroform upon liver cells, tetanus toxin, and nervous tissue, etc.

Cramer considers that in vitamin A deficiency it is the local defences that are weakened, so that at first there is a local infection of these tissues, represented by the tears in the case of the eyes, and probably by the mucus in the case of the mucous membranes of the digestive and respiratory tracts.

According to S. B. Wolbach and P. R. Howe (1925) a breakdown of the mucous membrane takes place with the formation of a stratified, keratinising epithelium in place of the normal one. From these infected tissues bacteria enter the blood stream and are dealt with there by the normal general defences, such as the clumping of bacteria by platelets and by the formation of agglutinins. Kingsbury and Cramer found, for instance, that vitamin A-deficient animals have in their serum an agglutinin against *Bacillus coli*, indicating that coli bacilli have passed from the intestine into the blood stream and there elicited the normal reaction of defence. There is also a progressive diminution in the number of platelets, which are playing their part by attaching themselves to the bacteria and clumping them.

The changes which take place in the tissues during vitamin A deficiency has been found by Javillier and co-workers (1929) to be primarily connected with their content of lipoids and fatty acids.

During vitamin A deficiency in mice, the lipoid or alcohol-ether soluble fraction and the fatty acids of the tissues were reduced while the cholesterol remained normal. Analysis of the isolated organs of vitamin A-deficient rats showed a decrease in the proportion of lipoids in the skin, liver, and muscles, an increase in the brain and bone, while the spleen, lung, kidney, and heart were normal. A decrease in cholesterol was found in all organs except the skin, the decrease ranging from 5.6 per cent. in the lung to 24.5 per cent. in the kidney. A smaller fall in fatty acids and lipoid phosphorus was observed, except in the muscle and skin.

The metabolism of cholesterol has been shown by other workers to be influenced by vitamin A in the direction of an increased secretion of bile acids when vitamin A is added to the diet. Kimura (1928) has found that a hypercholesterinæmia, induced by giving 0.5 gram of cholesterol daily, disappeared more slowly in dogs on a diet rich in vitamin A than in control animals—a result which was apparently contrary to his expectations. The thymus and the testes undergo a progressive atrophy, while the thyroid, kidneys, and spleen remain unaffected.

XXVI. "ANTI-VITAMIN A."

It is suggested by Mellanby (1930) that there is a substance present in most cereals, especially in the embryo, which can be antagonised by vitamin A in the same way that the anti-calcifying substance in oatmeal and other cereals can be antagonised by vitamin D. The condition which it produces he calls "nervous ergotism"—a disease appearing in epidemic form occasionally in European countries where rye is the chief type of cereal eaten. In examining the central nervous system of animals, Mellanby (1926) found by Marchi's method a scattered degeneration of the spinal cord of such a nature as to suggest subacute combined

degeneration. Observing that it occurred most often in animals receiving much cereal, and also only in animals receiving a diet deficient in fat-soluble vitamins, Mellanby suspected that the degenerative lesions in nervous ergotism depended not only on the presence of ergot in the diet, but also on the absence of a protective agent of the nature of a fat-soluble vitamin, probably vitamin A or a closely associated vitamin. The protective agent was not vitamin D, for ergot itself was found to contain vitamin D. If, however, cabbage, which contains little or no vitamin D, or mammalian liver oil (rich in A but poor in D), was added to the diet, then ergot left the cord untouched. Other substances containing vitamin A, such as egg-yolk, butter, or cod-liver oil, also prevent nervous ergotism in dogs. Vitamin D, in the form of irradiated ergosterol, will not in itself prevent the pathological condition.

Mellanby considers that these results probably afford the explanation of the arbitrary way in which nervous ergotism develops in human beings. It develops in times of famine and distress because not only is the rye ergotised but poverty prevents the consumption of such protective substances as milk, eggs, and green vegetables. If, however, any individual has a good supply of vitamin A in the liver and other organs, the nervous changes will not be produced until these reserves are used up. It seems probable, therefore, that ergotism of the nervous system can be prevented in human beings by the inclusion in the diet of sufficient sources of vitamin A.

Mellanby later found it possible to produce the pathological condition by rye germ unaffected by the *Claviceps purpurea*. That is to say, that the nerve toxin in ergotised rye germ is present merely in exaggerated amounts as compared with normal rye germ. It has not proved possible, however, to obtain without fail the nerve degeneration by means of pure grain products in all dogs. Some litters have remained without obvious degenerative changes of the cord even when diet has included much wheat embryo and the vitamin A intake has been small. The negative results seem to depend on the litter of puppies and not on the specimens of wheat germ tested. What the explanation of this resistance in families is has not been ascertained.

Mellanby suggests that in some cases the maternal diet has been so good as to give the resistant litters a larger reserve of the protective substance (vitamin A) in their livers.

A very interesting suggestion also comes from Mellanby on the vexed question of the relationship between maize and pellagra. It will be seen later that while Goldberger (1928) and Underhill (1928) have described a condition in dogs (black tongue), which resembles pellagra, Goldberger found it curable by yeast, while Underhill found butter and carotin the best curative agents. Mellanby suggests that the protective factor against the toxic factor in cereals which produces pellagra may be either vitamin B₂ or vitamin A, or both.

XXVII. VITAMIN A IN CERTAIN FOODSTUFFS.

Although the vegetable kingdom is the primary source of vitamin A, the fact that it can be stored in the animal body, and especially in the liver, has made animal tissues the most constant and important source.

A. ANIMAL SOURCES—(1) Oils and Fats.

(a) COD-LIVER OIL.—In the case of the cod, the vitamin A, which is originally formed in a marine diatom (*Nitzschia closterium*), passes through two intermediate carriers—the plankton and small fish—before it reaches the liver.

Cod-liver oil was originally produced by the simple method of putting the livers into a tub, leaving them to putrefy, and skimming off the oil. This method produced an oil of rich golden brown colour, with a strong and objectionable taste and smell. Möller, in 1853, intro-

duced the method of extraction by steam. The oil thus produced is nearly colourless and has no smell, and only a slight taste.

The question whether modern methods of manufacture tend to destroy the vitamin A has been investigated by Drummond and Zilva (1922), who found that there was no evidence that the "steaming" method of production of cod-liver oil destroyed the vitamin A in the oil. Investigation in Norway showed that the cods' livers were removed from the freshly killed fish and were placed at once in vats, and the oil separated by low pressure steam (direct or indirect). The first fractions of the oil obtained, which are the only ones used for medicinal purposes, are nearly colourless, and the chief further refinement to which they are subjected is freezing to remove stearine. The latter fractions of the oil recovered by the "steaming" process are coloured, and a small amount of brown oil is still obtained by the "rotting" process. The authors conclude, however :

"After personal investigation of many factories, and careful interrogation of many sources of information, we came to the conclusion that very small and quite negligible quantities of crude 'brown' oils are refined to produce medicinal oils. Several processes for this refining have at one time or another been put forward in patents, but as far as we are aware none has proved of any commercial value on a large scale."

Experiments also were made to compare the vitamin content of the crude brown oils, of the colourless oils, and of oils prepared in the laboratory ; in the last case every precaution was taken to prevent any destruction of the vitamin. From these experiments Drummond and Zilva concluded :

"From our exhaustive investigations of the effect of the modern processes of the preparation of cod-liver oil on its vitamin value we have formed the opinion that the 'steaming' methods yield oils of as high, if not actually higher, potency than were yielded by the old and almost extinct 'rotting' process.

"Further, the modern methods of refining, if we exclude, as we justly may, the almost negligible amount of bleaching of dark oils which occurs, scarcely affect the vitamin value at all.

"In the light of these observations it is obvious either that the popular belief that the 'dark' oils are more valuable as medicinal products than the modern 'white' oils is erroneous or that such beliefs arose at a time when some actual difference existed."

Sixteenfold variations may occur in the vitamin content of different samples of cod-liver oil, although even the least active samples of cod-liver oil contain more vitamin than does butter. Drummond and Zilva believe that these variations are a reflection of changes in the diet or in the physiological state of the fish at different seasons. The authors further note that the livers of other fish yield an oil as rich in vitamin A as cod-liver oil, and that therefore there is no obvious reason why the livers of other fish than the cod should not be used for medicinal purposes. They further question the utility of removing the stearine from cod-liver oil as this substance is rich in vitamin A.

Poulsso's (1924) investigations into the composition of cod-liver oil showed that even after heating the cod-liver oil for 16 hours at a temperature of 98° C. its vitamin content was practically unimpaired, but bubbling air through warm cod-liver oil deprived it of its vitamin content in a few hours. Exposure to air at ordinary temperatures proved injurious, and when a thin layer of cod-liver oil was poured out on a glass plate it was found to be inert after 14 days. Time alone appears to have little effect, and one of the most vitamin-rich samples examined was one which had been bottled for 3 years. With regard to prescribing cod-liver oil in the form of an emulsion it should be noted that, the oil being distributed in small drops, more of its surface is exposed than under ordinary conditions, and thus the chances of air contamination and loss of vitamin are considerably enhanced. As emulsions contain only 35 to 45 per cent. of cod-liver oil, they must be given in much larger doses than pure cod-liver

oil. The liver oil of certain other fishes besides the cod was found to be as rich in vitamin A as cod-liver oil. The liver of the male cod contains more oil than that of the female, and is just as vitamin-rich.

A somewhat acrimonious discussion as to the relative potency of different specimens of cod-liver oil has been carried on between Poulsson and Drummond. The question arose from Drummond's investigations in 1924 into the relation of the vitamin A potency of the liver oil to the sexual condition and age of the cod. Drummond came to the conclusion that no definite relationship existed, and that even the liver oils from spent cod in March fish, which must have spawned just before being caught, and which had no time to recuperate, did not deviate in potency from the oils of fish before spawning, and several months after spawning and consequently after recuperation.

In 1925, however, Drummond stated that the oils produced in Lofoten (Norway) were often relatively poor in vitamin because the cod were obtained in the Lofoten area during their spawning season, when much of the vitamin was being transferred from the liver to the reproductive cells. Poulsson investigated large numbers of samples of oil from Lofoten obtained from fish caught during the spawning season and found them to contain abundance of vitamin, and therefore challenged Drummond to explain the discrepancy in his results.

Drummond stated that he believed the cause to be found in the differences between the techniques employed, and laid emphasis on the desirability of making comparisons of the vitamin A potency of oils on the basis of the doses required to restore the same rate of growth. He suggested that the colorimetric determination was a more reliable method of assay of vitamin A than the biological test, and stated that he was now satisfied that the chief cause of the variation in vitamin value of the liver oils was probably the quantity and quality of the food of the cod. The most recent investigations of Drummond and Hilditch (1930) support the conclusion that the liver oils of Newfoundland cod are the most potent, those of Icelandic and Scottish origin the next in potency, those from Norway being the least potent.

The German cod-liver oil emulsions, known commercially as "Kraftin" and "Pentosin," are reported by Brigl and co-workers (1927) to be good sources of vitamin A.

Cod-liver Oil and Delumination.—Cod-liver oil is normally very brightly fluorescent under ultra-violet rays, but, according to the observation of P. R. Peacock (1926), its exposure to any source of light of sufficient intensity results in the following changes: (1) The normal bright golden fluorescence of the oil progressively disappears, this effect being termed "delumination." (2) Before full "delumination" occurs, the oil fails to give the arsenic chloride test for vitamin A. (3) Fully "deluminated" oil, when kept for some months in the dark, slowly regains much of its fluorescence, but the vitamin A appears to be permanently destroyed. (4) There is a change in the absorption spectrum corresponding with change in the fluorescence of the oil. As "delumination" proceeds, so the transmission spectrum extends into the ultra-violet region; as fluorescence returns, there is a proportionate return of the absorption spectrum.

Peacock emphasises the fact that light is a variable factor which must be taken into account in all quantitative experiments concerning the growth-promoting properties of cod-liver oil and the manner in which such oil is stored; secondly, to call attention to the unusual nature of the complex reaction of cod-liver oil to light—a reaction which is only partly reversible.

(b) OTHER FISH OILS.—The livers of other fish, such as pollock and haddock, contain, according to Drummond (1918), as much vitamin A as the cod-liver. According to Rosenheim and Webster (1927) the chromogen giving the AsCl_3 test for vitamin A is present in the livers of all animals, fish, birds, and mammals, while growth tests show the presence in salmon and halibut livers to one hundred times the extent in cod-liver oil. The extraction of the oil is, however, more difficult.

Salmon oil is, according to Davis and Beach (1926), a good source of vitamin A. The daily administration of 1 c.c. of salmon oil to each of ten birds suffering from vitamin A deficiency resulted in the recovery and increase in the weight and egg-production of all.

Ratfish liver oil is stated by Norris and Danielson (1929) to be a good source of vitamin A.

According to J. Malcolm (1929) the dried flesh of a fatty New Zealand fish (*Chilodactylus macropterus*) gave some evidence of vitamin A when fed at a level, per rat per day, of 1.4 gram containing 0.3 gram fat. Ether-extracted fats (Soxhlet) of the same, of oyster, and of red cod were useless.

A difference in the vitamin A content of the flesh oil and the liver oil of the salmon is reported by Schmidt-Nielsen (S. and S.) (1929). They state that the oil from the flesh prepared by boiling in water contains vitamin A corresponding to 20 to 30 units and vitamin D corresponding to 25 to 50 units. The liver oil, by extraction with ether, gave 600 and 1000 to 1200 units respectively. The colour reaction with SbCl_3 , calculated for 0.04 c.c. liver oil by the Lovibond system, was twenty times that of cod-liver oil. They also state that kippered herrings are a good source of vitamin A.

(c) OLEO OIL AND OLEO STEARIN.—The oil obtained by pressing rendered beef fat at a temperature approximating 90° F. is called "oleo oil," the solid residue being called "oleo stearin." Various grades are sold commercially; these are usually labelled No. 1, No. 2, No. 3, and "yellow oil," which is derived from grass-fed cattle. The vitamin A content of various samples of these oils and stearins has been tested by R. Hoagland and G. G. Snider (1926) by feeding experiments with rats. They obtained results confirming those of Halliburton and Drummond (1917) and of Osborne and Mendel (1914) to the effect that appreciable amounts of vitamin A were present in oleo oil. Yellow oleo oil was found to be the richest in vitamin A; No. 2 and No. 3 oils came next, and No. 1 oil the poorest of the oils. Rations containing 10 per cent. of the best oils caused normal growth in rats, while 20 per cent. in the case of the poorest oils did not suffice for normal growth. The oleo stearins contained considerably less vitamin A than the oils, the "yellow" oleo stearin being the richest. Although yellow oleo oil is richer in vitamin A than other grades of oil which were lighter, apparently there is no constant relation between the colour and its vitamin content. Comparisons with other commercial edible fats and oils prove that oleo oil ranks below butter but ahead of lard and vegetable fats and oils in vitamin A content.

(d) "MUTTON BIRD" OIL.—Carter and Malcolm (1927) have found some specimens to contain abundance of vitamin A, while Malcolm observes that the oil from the stomach of the young birds (*Astrelata lessoni*) has a high content, 0.04 gram, and in some cases even 0.01 gram, causing rapid growth and cure of xerophthalmia in rats. Rosenheim and Webster (1927) find that it gives the arsenic chloride colour reaction approximately to the same degree as cod-liver oil, and its content of vitamin A, as determined by feeding tests, runs parallel to the colour value. The oil has been examined chemically and is not a glyceride but a liquid wax of similar composition to sperm oil. The oil obtained from the stomach of the mutton bird, a species of petrel, consists largely of cetyl oleate and other esters of the higher alcohols. These esters can be broken down *in vitro* by mammalian pancreatic lipase and can also be absorbed from the alimentary canal of cats and rats.

(e) LARD.—Many differences have been reported as to the vitamin A content of lard. Halliburton and Drummond stated in 1917 that while rats would grow at a slow rate for a short time on a diet in which the only source of fat-soluble vitamins was lard, but that to save the life of the animals the addition of some other source of fat-soluble accessory substances was necessary.

Daniels and Loughlin (1920) have been widely quoted in their report that rats fed on a

ration from which all of this fat-soluble vitamin had apparently been removed except that which was in the lard, "grew normally, reproduced, and reared young." As these authors frankly state their results are out of harmony with the published results of other workers, and if there was no error in their experiment, the conclusion to be drawn must be that some samples of lard may contain some amount of fat-soluble vitamins.

Drummond and co-workers (1920), investigating the nutritive value of lard and lard substitutes from the standpoint of vitamins, was able to shed some light upon these differences. In their work, young pigs were brought up on diets some of which were practically devoid of vitamin A, while others were supplied with an ample amount of this substance. After several months on the various diets, certain animals from each lot were slaughtered, and samples of the leaf fat (from which the best lard is prepared) were taken. These were tested for the presence of vitamin A with the result that it was found that the leaf fat of swine fed on diets adequate in vitamin A contained that substance (thus the result of Daniels and Loughlin), but that its presence could not be demonstrated in the fats derived from those animals which were fed on diets deficient in fat-soluble vitamins. Drummond showed that it is possible to prepare lard from the leaf fat of hogs which have been fed upon a suitable diet, and to obtain in that lard vitamin A, but that an active sample of hog fat after being converted to lard by the rendering process commonly used in manufacture, employing high temperatures and aeration, lost its potency. It appears then that until, or unless the manufacturers of lard revise their process of preparation, no lard can be expected to supply any significant quantity of fat-soluble vitamins. Even if the manufacturers were to change their process of preparing lard, we could then be sure of no significant or constant quantity of vitamins in this substance unless adequate attention were given to the diet of the hogs from which the lard is to be prepared. It does not seem likely that at this time such attention to the preparation of lard is likely to be given. One may conclude safely, therefore, that lard, although an edible fat important in cooking, is an insignificant and negligible source of vitamin A in the diet.

Effect of Lard on Burr's "New Deficiency Disease."—A disease in rats reared on a diet deficient in fat has been described by Burr and Burr (1929). The outstanding effects due to the lack of fat in the diet are: (1) necrosis of the tail and a general scaly condition of the skin accompanied by hæmorrhagic lesions; (2) failure to grow to normal size, with a later decline in weight and death; and (3) abnormality of the kidney which permits the excretion of blood and protein in the urine. Burr states that lard is both protective and curative for this disease. The addition of 10 drops of melted lard daily was found to afford complete protection, and to permit approximately normal growth over a period of 18 months, while the same quantity, added daily even after the animal has become weak and almost moribund, was completely curative. Thus the addition of about 2 per cent. of lard to a fat-free diet suddenly changes the whole metabolism. The glycerol and non-saponifiable matter was found to be ineffective, while the fatty acids afforded the same protection as whole lard, Burr considers, therefore, that the effect must be attributed either to the well-known fatty acids of lard which have been almost 100 per cent. accounted for or to an undiscovered acid which occurs in traces.

The beneficial effect of the inclusion of fat in the diet has also been observed by McAmis, Anderson, and Mendel (1929); whether this effect of fat is due to its content of vitamin A or other vitamins, or to its action as a vehicle for the fat-soluble vitamins, or whether fat *per se* is essential, is not conclusively demonstrated.

(2) **Animal Tissues**—(a) **BEEF, PORK, AND LAMB.**—The vitamin A content of samples of beef, pork, and lamb was tested by Hoagland and Snider (1925) by feeding young rats on experimental diets in which the dried lean meat being tested furnished the only protein

fraction and was the only possible source of vitamin A. The meat samples were freed from visible fat, ground up with water, and dried in an oven at 60° C. for 20 to 24 hours.

Ten samples of beef were tested. None of the young rats which received 15 or 30 per cent. of dried beef in their ration grew normally, and most of them developed xerophthalmia. When the proportion was raised to 50 per cent. the rats grew quite satisfactorily, and when they received 95 per cent. their growth was nearly but not quite normal.

Seven samples of pork were tested. This meat contained distinctly less vitamin A than the beef. Fair growth was obtained in some animals by feeding 30 to 40 per cent. of dried pork, but even 95 per cent. did not induce very good growth, and the animals receiving this amount eventually developed ophthalmia.

Six samples of lamb were tested. Great variation in the vitamin content was found in the several samples; 30 per cent. of dried lamb in the case of three of the samples failed to produce good growth, whereas 20 per cent. in the case of two of the samples produced almost normal growth.

(b) LIVER.—The content of liver of animals has been found by Laquer and co-workers (1928) to vary with the season of the year, being much greater in autumn than in winter and spring. The liver of the pig contains very little vitamin A compared with beef liver, which is rich in it. The liver fats of herbivorous animals have been shown by Rosenheim and Webster (1927) to be twenty to one hundred times as rich as cod-liver oil. The vitamin content of fat from imported New Zealand liver equals that of the home-killed animal. The liver of birds, such as grouse and fulmar petrel, are also potent.

(c) POULTRY.—Hoagland and Lee (1926) have tested the relative amounts of vitamin A in the flesh and fat of poultry by feeding young rats. The birds' muscle was dried in air at a temperature not exceeding 60° C. and was powdered and stored at 4° C. The fat was rendered at a temperature of not more than 75° C. for less than one hour and was also stored at 4° C. The rats grew comparatively well on rations containing as the sole source of vitamin A 25 per cent. of dried Muscovy duck flesh or 30 per cent. of one sample of dried guinea-fowl flesh. Chicken, goose, and turkey flesh and other samples of duck and guinea-fowl flesh contained little or no vitamin A. The poultry fat contained more vitamin A than the flesh in each instance. One sample of goose fat and one of chicken fat produced quite good growth.

(d) FISH.—According to Drummond (1918), lean or "white fish," such as cod and haddock, contain comparatively little vitamin A, while "fat" fish, such as herring and salmon, are rich sources.

(e) SHELL FISH.—Studies by Jones, Nelson, and Murphy (1928) have shown that shell fish, such as clams and oysters, are comparatively rich in vitamin A, though it is found in greater concentration in oysters than in clams, and in the soft shell variety of clam than the hard. Fresh Chesapeake oysters were frozen and ground to a homogeneous consistency, and fed to albino rats. Two grams (0.03 gram on a dry basis) furnished sufficient vitamin A to cure rats of xerophthalmia. Dehydration at 40° C. under reduced pressure (10 to 15 mm.) was found to inactivate to a certain extent the vitamin A in oysters. According to Malcolm (1929) the vitamin A content of oysters in New Zealand is lower in the winter months of June, July, and August than in March to May, and showed a marked increase in September. No clear relationship was found between chemical composition and vitamin A value. Both the spawn and the spawned oyster contained considerable amounts of vitamin A, as did also tinned oysters.

(3) Milk and Milk Products.—Cramer (1927), discussing the problem of providing an ample vitamin A diet so far as it concerns Great Britain, points out that it mainly resolves itself into the production and consumption of a sufficient supply of milk and butter. "While it is known that the cow does not synthesise vitamin A, but produces it from the

food, and that there is undoubtedly an abundance of vitamin A in nature, nevertheless the fact remains that the main source for human beings of vitamin A is in milk and butter, since it is well provided in a palatable form, easy to handle, and in milk is combined with a protein of high biological value and essential mineral constituents, which are as a rule more deficient in other varieties of foodstuffs."

(a) MILK.—Milk was one of the first substances whose connection with vitamins led to the modern development of the whole subject; Lunin's statement (1881) that "other substances indispensable for nutrition must be present in milk besides caseinogen, fat, lactose, and salts," being followed up by Osborne and Mendel's (1914–1918) experiments on "protein free milk," and Stepp's discovery (1911, 1912) that milk and egg-yolk remedied the deficiency produced by extraction of foodstuffs with alcohol and ether. The vitamin A is present in the fat of the milk. Platon's experiments (1927) on the growth of young rats have shown that skim milk is practically devoid of vitamin A. Drummond and co-workers (1921) have investigated the relation between the diet of the cow and the vitamin A content of the milk, and have confirmed statements by other workers that the milk secreted by a lactating female will tend to be deficient in vitamin unless her diet contains adequate amounts of these factors.

The results of examinations of milk from different breeds were not numerous enough for conclusions to be drawn, but the general impression gained was that milk obtained from Jerseys and closely related breeds tended to be richer in vitamin A than that yielded by other breeds. No appreciable variation was found in the vitamin A content of milk at different seasons of the year apart from that associated with the different character of the diet ordinarily consumed at those seasons.

Under normal conditions colostrum appeared to be considerably richer in vitamin A than the later milk, which these workers are inclined to regard as an indication of the mobilisation of reserves of the mother. Partial mobilisation also had been shown to take place of the lipochrome pigments of the mother's body fat for the production of colostrum, which normally contains a much higher concentration of these colouring substances than the fat of later milk. There appears to be a gradual fall of vitamin A in the secretion as the colostrum is gradually replaced by normal milk.

Golding and Zilva (1928) have found that the presence of kale in the diet or the addition of cod-liver oil increased considerably the amount of vitamin A in the cow's milk, and Ethel M. Luce (1924) emphasises the fact that diet, rather than sunlight, is the principal factor in raising the growth-promoting factor in the milk. Similar results have been obtained by Chick and Roscoe (1926), who found that the vitamin A content of the milk reached its maximum when the cow received fresh green fodder in a dark stall and was not further increased by turning the cow out to pasture.

Sopp (1924) reports that milk in the spring-time contains less vitamin A than in other seasons. As soon as winter feeding in Norway gives way to pasture feeding the vitamin content of the milk begins to increase.

(b) PASTEURISED MILK.—It has been deduced by Schmidt-Nielsen (1929) from somewhat indirect evidence that pasteurisation at 63° C. destroys its vitamin A content. Neither xerophthalmia nor cessation of growth was taken as a criterion of vitamin A deficiency; it is stated merely that feeding of milk pasteurised at 63° C. to mature rats resulted in early death or diminished vitality of the offspring. It has been established that vitamin D is unaffected by pasteurisation, and since vitamin C was provided in the food, the condition was evidently due to lack of vitamin A. According to Dutcher and co-workers (1927), however, pasteurised milk retains its vitamin A content.

(c) EVAPORATED MILKS.—The vitamin A content of different preparations of evaporated milk has been compared with that of an equivalent quantity of the original raw milk. Dutcher,

Honeywell, and Dahle (1927) found that better growth in rats was obtained with the fresh milk in every case. In those experiments in which the rats received 1 c.c. of milk per day, the average weekly growth on vacuum evaporated milk was 65 per cent. of that produced by fresh milk. After fractional sterilisation it was reduced to 56 per cent. The corresponding figures for evaporated milk prepared by aeration were 38 and 34 per cent. respectively. The rats receiving 1 c.c. of fresh milk grew 3 grms. per week, while 1.5 c.c. of fresh milk increased the growth to 6 grms. per week, and maintained the animals in much better health. The same relationship between the evaporated and fresh milk was observed on feeding at higher levels (1.5 c.c. and 3.0 c.c.). It is concluded that evaporated milks made by the vacuum process have lost some of their nutritive value during the manufacturing process, and that this loss, which does not appear to be unduly great, is probably due to destruction of vitamin A. Aeration and sterilisation increase the destructive effect to some extent.

(d) **TINNED MILK.**—According to Donath (1929) the vitamin A content of tinned milk is in most cases satisfactory, little loss having taken place during the various processes of manufacture of the products. The difference in absorption of vitamin A in fresh milk and artificial milk has been pointed out by Amy L. Daniels (1926), who found that the fæces of infants receiving a milk dextri-maltose mixture contained vitamin A, while the fæces of infants fed on breast milk with cod-liver oil and orange juice lacked it. She concluded, therefore, that the vitamin A from fresh milk is more completely absorbed than is that from artificial milk.

(e) **HUMAN MILK.**—Vitamin A has been found by Macy and Outhouse (1928) in human milk in approximately the same concentration as in cow's milk.

(f) **MALTED MILK** is stated by Quinn and Brabec (1930) to be a rich source of vitamin A.

(g) **BUTTER AND MARGARINES.**—It was stated by Drummond, Coward, and Watson in 1921 that milk has a superior value as a source of vitamin A over the same quantity of milk fat in the form of butter. They found that rats fed on a supplement of 2 c.c. per day of fresh milk resumed and maintained almost normal growth. The rats to which approximately the corresponding amount of milk fat in the form of butter was given, showed some growth (or none) for 2 weeks, after which they ceased to grow, and declined. Those fed on a supplement of separated milk (0.07 per cent. of fat) showed some growth, but not nearly so good as that given by the same amount of fresh milk, and, later, growth ceased. The same result was obtained with those fed on a supplement of butter-milk.

The lower vitamin A value of butter, as compared with that of milk, was confirmed by the statement of Appleton (1921), that the diet of the inhabitants of Labrador is more completely supplied with fat vitamins from condensed and evaporated milks than from the butter available there, which is made from the milk of cows fed on poor hay.

Recent experiments by Crawford, Golding, Perry, and Zilva (1930), however, indicate that the vitamin A in milk is associated entirely with the fat, and that none is present in the milk other than in the fat. By testing the residual fat in separated milk and in the butter-milk after the cream was churned, they found that growth was more or less proportional to the residual fat. They state that there is no appreciable loss of vitamin A due to separating or churning.

Rosenheim and Webster (1927) state that butter has a vitamin A content $\frac{1}{10}$ to $\frac{1}{20}$ th that of cod-liver oil. Samples of butter were found to show a wide variation in the amount of vitamin A present, and, in comparison with some other fats, butter is not to be regarded as possessing so high a potency as was at first believed.

Butter produced during the winter months from stall-fed cattle on dry feeds of hay, roots, and cake has a low vitamin value; even the drying up of pasturage during a hot summer may lower its value.

The storage and preservation of butter does not appear to lower the vitamin A value if undue exposure to air is prevented and other conditions of storage are good. When butter is so exposed that damages of an oxidative character take place, rancidity is accompanied by comparatively rapid loss of vitamin A. Loss also occurs during the "renovation" of rancid butter if the methods used are likely to cause oxidation. These observations have been confirmed by those of Storms (1927) and have confirmed those of Steenbock, Boutwell, and Kent (1921), who suggest that the rations on which the butter fat has been produced may be responsible for variations.

(h) OLEO-MARGARINE.—The term "oleo-margarine," scientifically speaking, refers to a mixture of fats emulsified to give a suitable texture, containing at least some portions of oleo-oil, the low melting-point fraction of beef fat. On account of the nature of the diet of the animal, its body fat must contain some fat-soluble vitamin, and this has been found to be true by Halliburton and Drummond (1917), confirming previous work of Osborne and Mendel that a sample of oleo-margarine tested by them was found to be equal to butter fat in the nutrition of young rats.

McCollum and co-workers (1917) also found oleo-margarine to be satisfactory in growth-promoting qualities. The vitamin power of the oleo oil must be expected to show some variation, the effects of processing and rendering the oil, and storage, tending to lower the content.

An interesting fact in connection with the vitamin A content of margarine has been revealed by the researches of Morgan and MacLennan (1928) on the fluorescence of vitamin A containing fats. They have found that butter can be distinguished from margarine by the characteristic fluorescence which is observed when a beam of light falls upon it in a darkened room. Butter shows a yellow fluorescence and margarine a blue. When sufficient unsaponifiable matter from cod-liver oil is added to the margarine to bring it up to butter fat in vitamin A potency, the margarine exactly matches the butter fat in fluorescence.

The amount necessary, however, to make the margarine fluorescence approximate to that of butter is about five times that needed to equalise their vitamin A content. The difference in fluorescence cannot therefore be accounted for solely by the difference in vitamin content.

(i) GHEE.—Ghee is the clarified fat of cow's or buffalo's milk and is an important constituent of Indian dietary.

Ghose (1922) investigated its vitamin content and found some vitamin A present. Later investigations by Thomas (1927) and Bacharach (1930), however, indicates that ghee, as found in the open market, is for practical purposes devoid of vitamin A. Bacharach suggests that the discrepancy may be due to the fact that Ghose's sample was the result of an atypical process of manufacture, and also that the test was made before vitamin A was distinguished from vitamin D.

(j) CHEESE.—A study of three types of cheese was made by Agnes Fay Morgan (1926) to determine the retention of vitamin A activity in the finished product—American Cheddar, Limburger, and Swiss (Emmenthaler) being chosen. Tests were made on rats which had been depleted of vitamin A reserves. The animals were fed the usual basal diet described by Morgan, dry brewery yeast being fed as the source of vitamin B in 0.5 gram daily doses. Full growth was obtained in the cheese-fed rats, even in those which showed ophthalmia; so it was assumed that sufficient antirachitic material was supplied by the cheese and no irradiation was conducted. The results of the test showed that rats suffering from vitamin A deficiency recovered from ophthalmia and made normal growth with the addition to their diet of $\frac{1}{2}$ gram daily of portions of California cream cheese (Cheddar), or of Limburger cheese (from New York). These cheeses appear to retain in an unusually concentrated form the vitamin A of the milk from which they are made. In similar circumstances, 0.5 gram doses of Swiss cheese

(from Switzerland) did not cure ophthalmia or restore growth. With 1 gram doses, growth was normal, but eye disease persisted to some extent. Morgan suggests that the deficiency of this cheese may be due either to the relatively long heating and curing processes used or to selective bacterial action.

(4) **Eggs.**—The yolk of fresh eggs has been shown by several investigators to be rich in vitamin A, and the amount present has been found to be dependent upon the ration of the hens, the season of the year, and the methods of preservation. Bethke, Kennard, and Sassamann (1927) fed the yolks of the eggs from four pens of hens to rats which had exhausted their store of vitamin A. The yolks derived from a pen given two parts of cod-liver oil in the basal diet, and from a pen having access to a blue grass range, contained five times the effective quantity of vitamin A found in the yolks of eggs from hens fed on a basal diet alone, or allowed to eat alfalfa hay *ad libitum*. All these hens (White Leghorns) were confined indoors except the pen with access to a blue grass range. Egg-yolks from the pen receiving alfalfa hay were slightly, but not markedly, superior to the yolks from the pen on the basal diet alone. This result suggests that green grass is more potent in vitamin A than the product made into hay.

Manville (1926), using the yolk of eggs extracted with ether, and adding it to a basal diet deficient in fat-soluble vitamin, found that the eggs that had been kept in cold storage for one year had depreciated in vitamin value to an extent not less than 75 per cent.; those kept in water-glass for 18 months had depreciated by about 50 per cent., while frozen eggs had lost less than 50 per cent. of their vitamin value. During the course of the work it was found that an excess of egg-yolk extract could be as harmful to the rats as a deficiency. The optimum amount of egg-yolk extract was found in these experiments to be about 2 per cent. by weight of the total diet, but the amount varied according to the season of the year at which the eggs were laid; spring eggs contain considerably more vitamin than winter ones.

Chinese preserved ducks' eggs, "pidan," are reported by Tso (1926) to be as rich in vitamin A as fresh ducks' eggs.

(B) **VEGETABLE SOURCES OF VITAMIN A**—(1) **Oils and Fats.**—Vegetable oils in general have been found to be low in their content of vitamin A because there is very little of the vitamin in the seeds from which the oil is produced. McCollum and co-workers (1917) have reported that cotton-seed oil and linseed oil are deficient in growth-promoting properties, Osborne and Mendel (1914) similar findings from almond oil, while Halliburton and Drummond (1917) have corroborated these statements.

Drummond and Zilva in 1922 carried out an interesting research on the subject. They first investigated the vitamin content of the oil-bearing seeds; they found that linseed contained a fair amount of vitamin A, but that in seventeen other varieties of seeds examined there were at most only traces of this vitamin. Though most of the vitamin A present in the seeds passed into the crude oil, the quantity might be less than one-tenth the vitamin A content of butter. The only exception was crude palm oil; highly pigmented specimens of this oil in some cases had a vitamin A content equal to that of butter; unfortunately it was found impossible to render the crude palm oil palatable by any process which did not involve destruction of the vitamin.

Stammers (1921) found that linseed oil, prepared cold, and drawn from whole seeds contained a little vitamin A, but arachis oil, prepared in the same way, contained none. Maize oil, however, both in the refined and the crude, rancid state, is reported by Stammers to have a high content of vitamin A.

(a) **WHEAT OIL.**—Wheat oil has been chiefly investigated for its vitamin E content, but Sure (1928) finds that it contains also appreciable amounts of vitamin A. The wheat oil was administered to young rats which had developed ophthalmia on a vitamin A-deficient

diet. The dosage given was 0.01 c.c., 0.05 c.c., and 0.1 c.c. per animal per day. In a dosage of 0.01 c.c. per day the results proved that this amount is not comparable with 0.01 c.c. of cod-liver oil in vitamin A content. Better results were obtained with 0.05 c.c., and 0.1 c.c. was found completely curative in cases of advanced ophthalmia.

(b) OLIVE OIL.—According to Javillier and Émérique (1929), unrefined olive oil contains vitamin A in small amounts but sufficient, in doses of 15 per cent. of the diet, to protect against xerophthalmia in rats, and to maintain normal growth. The various processes to which the oil is subjected, however (neutralisation, deodorisation by super-heated steam, etc.), during the refining processes, combine to destroy the factor, and the refined oils reach the market with varying amounts of the vitamin, usually too small to detect by physiological experiment.

(c) CORN OIL.—Crude corn oil in doses of 1.5 to 1.7 gram daily was found by Meyer and Hetler (1929) to cure ophthalmia in rats, but refined corn oil was deficient in vitamin A, owing probably to the result of heating in purification.

(d) NUT MARGARINES.—Although vegetable fats and oils, such as are used in the preparation of "nut margarines," are likely to be deficient in fat-soluble vitamins, some samples show considerable potency. Ordinarily the only reliable vitamin content of margarines of this type will be that imparted by the milk in which they are churned, and by the relatively small amount of butter incorporated in them by manufacture. Recently certain brands of margarine have been prepared in which the deficiency of fat-soluble vitamins has been made good by the process of incorporating a vitamin concentrate during the manufacture. Some of the margarines have been tested by K. H. Coward (1928) on behalf of the Pharmaceutical Society of Great Britain.

The assay of vitamin A was based on the response given to small doses of the butter or margarine by animals which had become steady in weight on a diet deficient only in vitamin A. A special feature of the society's vitamin A free basal diet is that it is rendered completely antirachitic by irradiating it in very thin layers under a mercury-vapour lamp. It is thus assured that cessation of growth is not due to a lack of vitamin D, and any response in growth when butter or margarine is given is due to the vitamin A content of the substance under examination.

The results showed that these margarines were equal to the best summer butter in their vitamin A content.

(2) Cereals.—Cereals are not on the whole a potent source of vitamin A. The vitamin has been shown by Steenbock and Coward (1927) to be contained in the endosperm rather than the embryo.

(a) CORN.—Yellow corn is richer than white (Steenbock and Coward, 1927), and according to Russell (1930) richer by 50 per cent. than the white-capped yellow variety. Russell also states that the concentration is greatest in the nitrogenous outer layer of the endosperm.

Biological tests by Hauge and Trost (1928) showed that vitamin A was always associated with the yellow endosperm and lacking in corn grains of pure white endosperm, even when grown on the same ears as the yellow grains. It appears that there is a close physiological association between vitamin A and yellow endosperm colour character in dent corn.

(b) CORN-MILLING PRODUCTS.—A study of the distribution of vitamin A in yellow corn has been made by Meyer and Hetler (1929). The milling products obtained in the wet milling process of corn-starch manufacture include starch, corn germ, corn-germ meal, crude corn oil, gluten, bran, etc. Their experiments, based on the cure of ophthalmia in young rats fed on a vitamin A-deficient diet, indicated that the vitamin was contained in the gluten, but that some loss occurred in the separation.

(c) **WHEAT.**—According to Steenbock and Coward (1927), wheat germ is efficacious as a source of vitamin A if fed in the proportion of 20 per cent. of the diet.

(d) **FLOUR.**—Both brown and white flour are poor sources of vitamin A, but Sheehy (1927) has shown that brown flour contains more than white.

(e) **MAIZE.**—It was originally shown by Steenbock and Boutwell (1920) that white maize contained much less vitamin A than the yellow variety. This observation has been confirmed by Jansen and Donath (1928) in varying types of maize from the Indies, white maize being shown to be lacking in vitamin A.

(f) **OATS.**—According to Steenbock and Coward (1927), and Bezssonoff (1929), oats are very poor in vitamin A.

(g) **BARLEY (GERMINATED).**—According to Schittenhelm and Eisler (1928), germinated barley in a dosage of 15 per cent. of the ration prevents xerophthalmia and induces good growth.

(3) **Vegetables.**—Sherman and his collaborators (1925) have investigated by a special method the vitamin A content of certain vegetables, including carrots, string beans, peas (raw and cooked), and lettuce. In these experiments, rats were used which were first freed of their storage of vitamin A by the following diet :

Vitamin A free casein	20
Starch	70
Dried brewer's yeast	5
Salt mixture	5

As soon as growth ceased on this diet the rats received varying amounts of the foodstuffs to be tested, and the amount necessary to obtain about 3 grms. per week for 8 weeks (24 to 25 grms. total grain) was used in comparison.

The following table shows the vitamin A values in some vegetables tested by this method :

Foodstuffs.	Amount necessary to produce an average of 25 grms. gain per rat in 8 weeks.				
Spinach	0·016–0·018
Carrots	0·040
Beans	0·275–0·300
Peas (raw)	0·500
Peas (cooked)	0·500
Lettuce	0·600–0·700

(a) **CARROTS.**—Yellow and red carrots, are, at any rate for poultry feeding, a good source of vitamin A, but White Belgian carrot, even when given in the amount of 16 grms. daily, did not in Davis and Beach's experiments (1926) prevent signs of vitamin A deficiency.

(b) **LETTUCE.**—The vitamin content of lettuce has been shown by Dye and Crist (1929) to vary with the conditions of its soil nutrition. Lettuce grown on soil with inorganic fertiliser (a mixture of KNO_3 , $\text{CaH}_2(\text{PO}_4)$, and HCl) was greener and more vigorous than that grown on soil without fertilizer or with organic manure, and its vitamin A content was correspondingly greater. It is suggested that the vitamin A content is associated with the degrees of greenness and not necessarily with vigour apart from differences in chlorophyll.

The suggestion receives further support from recent experiments (1929) by Kramer, Boehm, and Williams, showing the degree of difference in vitamin A content in outer green leaves and inner white ones. It was found that the green outer leaves of head lettuce contained thirty or more times as much vitamin A as equal weights of the white leaves, the unit for the dark leaves being between 0·015 and 0·02 gram, and for the white, 0·6 gram.

(c) MUNG BEAN SPROUTS, which constitute a considerable portion of the diet in the oriental population of Honolulu, have been investigated by Miller and Hair (1928). In determining vitamin A, a basal diet of meat residue, starch, yeast, salt mixture, sodium chloride, and Crisco was used, the meat residue was treated for extraction of casein, and the whole mixture exposed to bright sunlight to ensure the presence of vitamin D. Both raw and cooked bean sprouts were fed. Four grms. of raw and 4 grms. of cooked sprouts produced gains approximating 3 grms. Bean sprouts are a fair source of vitamin A compared to some other vegetables; according to Sherman's evaluation they have 110 to 120 units per lb. as against 750 to 3000 units per lb. for lettuce, and 2500 to 3500 units for green peas.

(d) PEAS.—Eddy and co-workers (1926) state that green peas are a rich source of vitamin A, especially young ones, maturity reducing their content. *Canned peas* have as high a content as the fresh, being unaffected by the process.

(e) SPINACH.—By extracting dried spring spinach with ether and acetone, evaporating off the solvents *in vacuo*, and drying over sulphuric acid in a desiccator protected from the light, Willimott and Wokes (1927) obtained an extract representing 0.45 per cent. of the fresh leaves containing only 1 per cent. of the mineral matter of the latter and free from oxidising enzymes (oxygenase and peroxidase). The extract was a potent and stable source of vitamin A, since 25 mgrms. fed daily to rats secured freedom from xerophthalmia and induced satisfactory though slightly subnormal growth.

Chick and Roscoe (1926), and Boas (1926), have also shown that fresh spinach leaves contain a large quantity of vitamin A.

(f) TURNIPS.—The White Globe turnip appeared to contain a little vitamin A, while the Swede turnip and three varieties of mangolds were regarded by Davis and Beach as valueless as sources of vitamin A for poultry.

(g) WATERCRESS has been shown by Coward and Eggleton (1928) to be a rich source of vitamin A, 0.1 gram only of the green leaf being necessary to promote normal growth in a rat which has become steady in weight on a diet lacking this factor only. Its growth-promoting properties are stronger in the spring and summer than in the winter.

(4) **Fruits.**—(a) BANANAS contain an appreciable amount of vitamin A. Eddy and Kellog (1927) estimate it at 56 units per ounce. (One unit of vitamin A, according to the U.S. Pharmacopeia, is that required to produce 25 grms. gain in 56 days in 30 days' old white rats, fed on a basal diet adequate in all known factors except vitamin A.) This is equivalent to 1 in 20 the vitamin A value of good butter.

According to the Sherman-Munsell (1925) method of vitamin A assay, bananas are, in spite of their high-water content, a better source of vitamin A than lettuce, at least five times as rich in it as apples, and the equal of green peas.

Sopp (1924), on the basis of the quantity of banana sufficient to cure xerophthalmia in rats (1 gram for every 50 grms. of body weight), calculates that a three-year-old child, weighing between 26 and 30 lbs., would require four or five bananas a day, but she adds that a much smaller quantity would probably be sufficient to provide the necessary quantity of A vitamins.

(b) GRAPE FRUIT.—According to Willimott and Wokes (1926), fresh grape fruit and the extract obtained with 90 per cent. alcohol contain small but definite quantities of vitamin A, but the oil from the rind, obtained by acetone extraction of air-dried material, was practically devoid of the vitamin in 0.1 c.c. doses per rat.

(c) ORANGES.—The vitamin A content of orange juice has been tested by Willimott (1928), using rats which had been fed on a diet free from vitamin A (vitamin D being supplied by irradiated cholesterol) until cessation of growth and onset of xerophthalmia occurred. The juice was obtained from ripe California oranges which were peeled before pressing to obviate

contamination of the juice by the oil known to be rich in vitamin A. The juice was filtered from cell tissue. It was found that 5 c.c. of orange juice daily was the minimum adequate curative dose for the rats.

(d) PEACHES.—According to Eddy and co-workers (1926), partially ripe peaches have a vitamin A content equivalent to one-third that of butter fat, and their potency increases as they ripen.

(e) STRAWBERRIES.—The conclusion of Kohman, Eddy, and Halliday (1928) from their experimental work is that strawberries are not highly endowed with vitamin A, being only one-fortieth as rich in it as tomatoes.

(f) TOMATOES.—Ripened tomatoes contain an appreciable amount of vitamin A, and green tomatoes a somewhat less amount (Morgan and Smith, 1928).

House, Nelson, and Haber (1929) have investigated the effect of artificial ripening by ethylene on the vitamin A content of tomatoes. The ethylene-air mixture, approximately 1 in 800, was passed over the tomatoes in a bell jar provided with inlet and outlet tubes. They concluded that the vitamin A content was practically unaffected by artificial, as compared with natural, ripening. More recent experiments by Jones and Nelson (1930), however, seem to show that naturally ripened tomatoes are a better source of vitamin A than artificially ripened, though they were working on tomato juice, not whole tomatoes. No material difference was observed in the vitamin A content of the green tomatoes picked at different stages of development, whether treated with ethylene or not. No indication was observed that the ethylene treatment had any harmful effect on the vitamins already developed.

Experiments on similar lines by Morgan and Smith (1928) suggest that carotin and lycopin have a protecting effect in the development of vitamin A in plants when exposed to sunlight.

(5) Tropical Foodstuffs—(a) INDIAN FRUITS.—Different fruits eaten by native Indian populations were tested by Jansen and Donath (1924) for their vitamin A content by observing their curative properties on early xerophthalmia in rats. A wide variation was shown, e.g. paw-paw contains a considerable amount of vitamin A; palm-kernel oil (from a type of fruit with a hard stone) contains none, whilst palm oil (from a stoneless species) is fairly rich in the vitamin.

(b) THE BATATA, which forms a staple article of diet in Central Java, was similarly tested. The yellow and orange-red types proved to be rich in the vitamin (the yellow being growth-promoting at a 20 per cent. level), whilst the white and purple species showed a marked deficiency, the purple batata being slightly superior. Other fruits tested by Jansen and Donath (1928) and found to contain vitamin A were grape fruit, bread fruit (0.5 gram daily dosage), Cashew apple, and mango (1 to 2 grms. daily dosage).

(c) AVOCADO.—The avocado has been shown by Weatherby, Youtz, and Watson (1929) to be a surprisingly good source of vitamin A. A daily dose of 2.5 grms. prevented xerophthalmia and maintained good growth, and the growth curves indicated that even less than 2.5 grms. would have been sufficient. The high oil content of the grade of avocado tested (the Calavo) is probably a parallel factor with its high vitamin A content.

(d) TARO, ETC.—For centuries certain foodstuffs, such as taro (*Colocasia antiquorum esculenta*), from which poi is made, limu eleele (*Enteromorpha intestinalis*), and limu lipoi have formed the staple diet of the Hawaiians, and have resulted in native physical excellence. They have all been found by Miller (1927) to contain vitamin A.

(e) WHITE YAUTIA, YELLOW YAUTIA, and PLANTAIN, which occupy an important place in the dietary of the people of Porto Rico and other tropical countries, were tested for vitamin A by E. J. Quinn and D. H. Cook (1928). The unit of vitamin A was taken as the amount

which, administered daily, would produce a gain in weight of rats of 3 grms. per week under standard conditions. Plantain was found to contain 10 units per gram and is thus relatively high in vitamin A content, containing somewhat more than the common banana, tomatoes, and sweet potatoes. Yellow yautia contained over 5 units of vitamin A per gram, while white yautia contained only 0.4 unit per gram. It is suggested that these results furnish further evidence to indicate a possible relationship between the colour of a food and its richness in vitamin A.

(f) OTHER ORIENTAL FOODS, Chinese persimmon, the flower of *Hemerocallis flava* Linnæus (huang hua ts'ai), *Glycine max* Linnæus (yellow bean curd), and Kan lu have been tested for their vitamin A content by Sherman (1929). The first two foods named were found to be rich in the vitamin, the yellow bean curd to contain a moderate amount, and the Kan lu only a small amount of the vitamin.

(g) SOME PHILIPPINE FOODS, including *Basella rubra* and the powdered leaves of *Capsicum frutescens*, are stated by Hermano (1930) to be good sources of vitamin A. *Vigna sinensis* (sitao) also contains some vitamin A, but is not so rich in it as *Basella rubra*.

(6) Honey.—The most recent investigations show that honey is not a good source of vitamin A. Neither English comb honey nor West Indian honey was found by Hoyle (1929) to cure vitamin A deficiency in daily doses of 2 grms.

XXVIII. VITAMIN D (HISTORICAL RÉSUMÉ).

The enunciation of a vitamin specific in the prevention of rickets and its separation from the growth-promoting, anti-xerophthalmic vitamin, apparently co-existing in the same foodstuffs, has provoked much discussion and involved complicated research. Following on Hopkins' pioneer work, published in 1913, Mellanby in 1919 published the results of his experiments on dogs, which demonstrated that in experimental animals in the laboratory rickets, or, at all events, a form of dystrophy of the bones indistinguishable from rickets, could be produced and controlled by dietetic factors alone, apart from other environmental changes.

Throughout the investigation the important criterion of rickets in the young dog was disturbed calcification in the growing bones, these changes being established by radiograms, by chemical analysis, and by histological evidence. The histological examination was the decisive one, and the crucial test was the presence of osteoid tissue.

The factors which produced the bony changes characteristic of rickets were given as follows: "A deficiency of calcium and phosphorus in diet; a deficiency of fat containing the antirachitic vitamin in diet; excess of bread, other cereals and carbohydrates; absence of meat; excess of the protein moiety of caseinogen free from calcium; confinement."

Mellanby found that the diet most favourable to the rapid production of rickets in a young puppy was one containing an adequate amount of protein (separated milk), an excess of carbohydrate (bread), a sufficiency of water-soluble B and C vitamins and of salt, but very defective in fat. By adding measured amounts of various animal and vegetable fats, and by observing the degree of rickets produced, he was able to estimate the antirachitic power of each variety of fat. The broad result was that animal fats were powerfully antirachitic; the vegetable oils were either feebly so, or destitute of this power. Now, experiments on young rats showed that the animal and vegetable fats fell into the same groups in respect of their power to promote growth, or, according to the vitamin hypothesis, in their content of fat-soluble A.

In spite of the discrepancies of this general statement, Mellanby was led to the conclusion that "the disease in each species, lack of growth in the rat, and rickets in the puppy, is probably due to the same vitamin, fat-soluble A." He found other points in favour of this identity, in the fact that in each animal the disease took several weeks to develop, and also that beyond a certain age in the young rat and in the puppy the respective diseased condition became

very difficult to produce. These were the points in favour of rickets as a vitamin deficiency disease, and of the identity of that vitamin with fat-soluble A shown to be essential for growth in rats.

Difficulties and objections to this interpretation soon arose. In the first place, the puppies with rickets did not cease to grow; on the contrary, the worst degrees of rickets were found in those with most rapid growth, while, if growth ceased, the rickety changes in bone were not produced. That is to say, it was to be understood that the same vitamin caused growth to cease in the young rat, while in the puppy it produced rickets but allowed growth to proceed. Then lean meat, a food poor in fat-soluble A for rats, was shown to possess definite antirachitic power for puppies—a fact for which no adequate explanation was found. Lastly, a few experiments were made with cabbage and extracts of cabbage, and these failed to prevent rickets and set up diarrhoea, whereas, in experiments in rats, green leaves had been shown to be specially rich in fat-soluble A. Each of these difficulties in itself needed special explanation, which was not forthcoming. Together they appeared to negative the acceptance of fat-soluble A as an antirachitic vitamin, and the Glasgow workers, under the direction of Paton and Findlay (1918), contended that since rickets could be prevented by economic conditions it was unnecessary to postulate the existence of any such accessory factor.

In *The Study of Social Economic Factors in the Causation of Rickets*, compiled by Miss M. Fergusson in 1918, the doctrine was developed that rickets could be prevented by fresh air and exercise, with massage as a part of the treatment, and the following were among the conclusions: "The evidence is against a deficiency of milk, of butter, or of the fat-soluble A substance being a determining factor. Inadequate air and exercise seem to be potent factors in determining the onset of rickets."

Shipley, McCollum, and co-workers, working on the same lines (1920–1922), showed that a deficiency of vitamin A alone, the diet being otherwise complete, did not produce rickets, and from their results concluded that vitamin A could not be excluded as a factor in rickets, but that it could not be regarded as the sole factor, probably having an indirect influence by its effect upon the metabolism of calcium and phosphorus.

Hess, Pappenheimer, and co-workers (1921), working simultaneously and independently, found that a diet which was high in its calcium content, but low in phosphorus, produced definite rickets in four weeks, and that the simple addition of 3 to 4 per cent. potassium phosphate sufficed to prevent its appearance. They stated further that the administration of cod-liver oil brought about a progressive recalcification of the ribs, but by submitting the oil to a process of continuous oxidation the anti-xerophthalmic properties were destroyed while leaving the antirachitic intact. From this they concluded that the antirachitic principle in cod-liver oil was not identical with the vitamin A encountered in food.

In 1920 Hess and Unger also concluded that vitamin A did not bear the same relation to rickets as did vitamins B and C to beri-beri and scurvy respectively.

Goldblatt and Zilva (1923) investigated the effect of heat in the presence of air on the growth-promoting and antirachitic properties respectively of cod-liver oil. In this experiment about a litre of cod-liver oil of known growth-promoting and antirachitic properties was heated in an oil-bath at a constant temperature of 120° C., whilst a continuous current of air was aspirated through it. Portions of the cod-liver oil were removed 6, 12, 18, and 24 hours after it had reached 120° C., and in these samples the growth-promoting and antirachitic properties were estimated. The results showed that the growth-promoting and antirachitic properties of cod-liver oil are inactivated at different rates by heat in the presence of air. They also investigated the quantitative distribution of the two factors, using spinach as the material to be tested. The fresh leaves were ground to a paste and administered in quantities from 0.1 gram to 3 grams daily to rats that had stopped growing on fat-soluble

deficient diet and to rats on a rickets-producing diet. The minimal growth-promoting dose was found to be between 0.1 and 0.25 gram, while even 3 grms. daily, the maximum dose which it was found practicable to administer (about 30 per cent. of the diet), did not have the slightest effect in promoting calcium deposition. The animals that received the spinach were as severely rachitic as the controls.

This result, showing the great disparity between the growth-promoting and the anti-rachitic potency of spinach, was similar to that of McCleendon and Schuck (1923), who found that from 0.1 to 0.5 gram of dried spinach cured ophthalmia, while it showed no evidence of affording protection against the development of rickets even when it formed as much as 75 per cent. of the daily diet. Zucker and Barnett (1923) also showed that an alcoholic and ether extract of spinach, while it possessed the power to promote growth, was unable to protect against the development of rickets.

The specific effect of cod-liver oil, however, had been earlier noted both by Hess and Unger (1917), working amongst negro children in the Columbus Hill district of New York, and by Schabadi (1919), Freund (1905), Schloss (1913), and Orgler (1912), with regard to its effect on calcium retention, though in the latter investigations there was no suggestion that its action was due to a vitamin.

By means of a histological test—the so-called line test—McCollum, Simmonds, Shipley, and Park (1922) showed that cod-liver oil caused a remarkable deposition of lime salts in the cartilage of the rachitic rat close to its junction with the metaphysis.

Park and Howland (1921) demonstrated also, by means of the X-rays, the deposition of lime salts in the bones of rachitic children following administration of cod-liver oil.

The discovery that ultra-violet light had definitely curative action in connection with rickets was an important advance in the study of the disease and a step forward in the reconciliation of the conflicting theories.

In 1919 Haldershtinsky showed by means of radiographic examination the curative process in cases of advanced rickets after 4 weeks' treatment with the mercury-vapour lamp, and his results were quickly followed by those of other observers. Erlacher (1921) treated 42 children from 1 to 7 years, including early, well-developed, and chronic examples of rickets. After 4 weeks' treatment the skiagrams showed an increased deposit in the osteoid tissue, and clinical improvement generally occurred after 6 weeks' treatment. Spontaneous fractures rapidly united, and osteotomies and osteoclases became consolidated with a firm callus in 4 to 6 weeks' time.

Jundell (1922), testing the comparative value of treatment of rickets by phosphorus and cod-liver oil with a reduced dietary, and by quartz lamp radiation, found the response in each case equally good.

In 1924 Hess instituted an inquiry into the nature of the light waves which possessed the power of curing or preventing rickets. For this purpose he used the mercury-vapour quartz lamp and various glass filters which were known to exclude waves of definite lengths, and came to the conclusion that the protective rays were in the ultra-violet zone, and were about $300\text{ }\mu$ in length or shorter.

The influence of both light and diet on the occurrence of rickets received indirect confirmation in the course of post-war work in Vienna by Chlek and co-workers (1923), where one group of children were kept free from or cured of rickets by being freely exposed to sunlight, and another by receiving a liberal milk ration, provided that the milk was obtained from cows that had been pasture fed.

Mellanby made a further attempt in 1925 to reconcile the light and dietary factors in the causation of rickets. Koronchevsky (1922), dealing with the problem of what can cause rickets under experimental conditions, had suggested a quantitative interrelationship between

an adequate supply of calcium and an adequate supply of the antirachitic vitamin in the diet.

Bearing in mind Grosser's finding that marked calcium retention occurred in rickety children when calcium was introduced subcutaneously in the form of a glycerophosphate, Korenchevsky and Carr (1925) injected a solution of Kahlbaum's calcium glycerophosphate, containing 14.72 per cent. calcium and 11.34 per cent. phosphorus, into the subcutaneous tissues of rats. These injections considerably increased the degree of calcification of the skeleton of rats kept on a diet deficient in the fat-soluble factor only. There was not, however, the same general improvement as would follow a course of cod-liver oil, for the water content of the bones of these rats was the same as or higher than that of the controls, and only in about 50 per cent. of the rats injected was a slight improvement in the rachitic changes in the skeleton found histologically. Korenchevsky therefore suggested that there was a difference in the mechanisms of calcification induced in the skeleton of rats on a diet deficient in the fat-soluble factor by administration of calcium glycerophosphate and of cod-liver oil respectively.

Mellanby now tried to follow up these results, especially with regard to the significance of the phosphorus and calcium elements, and to the importance of balance of constituents. He brought forward evidence (1925) to prove that some cereals have a strongly adverse influence on bone calcification, especially oatmeal, which contains more calcium and more phosphorus than the other cereals tested. He came to no definite conclusion as to the method whereby cereals produced this detrimental effect on bone, but suggested that absorption and digestion liberated some toxic substance. He concluded, however, that the amount of calcium and phosphorus in food was of but secondary importance in the control of the deposition of these elements in the growing bones, though the diet must, of course, contain a sufficient quantity of the salts. He suggested that the most important factor was the presence of the anti-rachitic factor in certain foodstuffs, particularly cod-liver oil, its action being to antagonise the "cereal action." The same effect could be produced to some extent by exposing the animal eating the cereal, or even the cereal itself, to ultra-violet irradiation.

In the meantime the calcium-phosphorus content of the diet in rickets had been receiving attention from many quarters. The outcome of the work of the American investigators, McCollum, Simmonds, Shipley, and Park (1922), and Sherman and Pappenheimer (1921), had been to show that the limitation of either calcium or phosphorus in the diet led to the failure of proper ossification, and Sherman and Pappenheimer more particularly showed that the form in which the phosphorus was given—that is, as a soluble inorganic phosphate rather than as an organic phosphate in various types of combination—was important. Telfer's work (1926) also suggested that the error of phosphorus metabolism in rickets might be of even greater importance than the disturbance in the metabolism of calcium, while Howland and Kramer (1921) had previously shown that the inorganic P_2O_5 of the plasma is generally reduced. Paton and Watson in 1923 opened a reinvestigation into the question, in the hope of reconciling the contradictory evidence, but apart from the fact that they disproved the infective theory of rickets, and confirmed the fact that cod-liver oil exercised a marked effect in favouring the cure of rickets, their results failed to clear up the situation materially. A curious statement, showing the difficulties under which investigators laboured, while the source of vitamin D still remained a mystery, was that "the addition of olive oil to a diet which was adequate to prevent rickets actually favoured its onset. This can hardly be explained by deficiency of the oil in some antirachitic substance."

In 1923 Hume and Smith had made the then unexplainable discovery that rats kept on a diet deficient in fat-soluble vitamin grew better if they were kept in glass jars which had been irradiated. It was not until after Steenbock and Black (1924), and Hess (1924),

working independently, had shown that experimental rickets could be prevented if the food given to the animals was subjected to ultra-violet light, and Drummond and co-workers (1925) had brought forward evidence indicating that certain sterols exposed to ultra-violet light acquired antirachitic properties, that it was realised that the sawdust in the glass jars might have played a part. Hume and Smith (1926) then devised experiments "to test whether antirachitic radiations of any kind are given off from irradiated sawdust, and, secondly, whether any volatile substance having antirachitic potency is given off." Calcium analysis of dry bones was used as a criterion of the presence of an antirachitic influence. When a quartz plate was interposed between the rats and the irradiated sawdust, a clearly negative result was obtained, thus disposing of the possibility that the sawdust exercises its action by means of secondary radiations which can penetrate quartz. While some of the other results proved difficult of interpretation, the evidence for the following statement was satisfactory: "irradiated sawdust exercises a beneficial action on growth and calcification when consumed by the rats."

These results were confirmed by Rosenheim and Webster (1926), who extracted sawdust with chloroform, and irradiated the resin so obtained for half an hour at a distance of 40 cm. under a Cooper-Hewitt lamp. They found that this extract administered in olive oil to rats on a Sherman-Pappenheimer No. 84 diet in mgrm. doses entirely protected them against rickets, as proved by radiographic and chemical evidence, and concluded that the increased growth observed in the experiments of Hume and Smith were due to the consumption of irradiated sterols in sawdust.

The connection between cholesterol and the antirachitic vitamin was gradually realised through successive investigations of independent groups of workers. It had been shown by McCollum and Davis in 1914, and by Steenbock and Boutwell in 1920, that the substances which both promote growth and prevent rickets could withstand saponification in alcoholic solution and could be concentrated without loss in the unsaponifiable fraction of such materials as cod-liver oil, provided that oxidation during extraction were prevented. Drummond, Rosenheim, and Coward (1925), however, though they discovered that about half this unsaponifiable material from cod-liver oil usually consists of cholesterol, established the fact that this substance was not responsible for the physiological effects of vitamins A and D. It occurred to Drummond, however, considering the general resemblance of the "cod-liver oil reaction" with sulphuric acid to those of cholesterol, that the active principle in question might be artificially produced from cholesterol by a slight chemical change, such as takes place under the influence of light. The idea was supported by the observation of Schulze and Winterstein (1904, 1906) that cholesterol, as well as phytosterol, when exposed to sunlight, becomes yellow, its melting-point falling by 20° to 40°, and further strengthened by Huldshinsky's discovery of the curative action on rickets of ultra-violet light. Still further confirmation was now received from two quarters: Steenbock reported in 1924 that exposure of foods to ultra-violet light conferred calcifying and growth-promoting properties upon them, while Hess (1924) also found that the unsaponifiable material of inactive oils became active after exposure to light. Drummond, Rosenheim, and Coward (1925) now confirmed Steenbock's observations on the action of light on foodstuffs, and found that while starch and proteins remained inactive, fat, containing 0.25 per cent. of a sterol, could be activated. They therefore proceeded to expose samples of cholesterol obtained from cod-liver oil and brain, and purified by recrystallisations, to a quartz mercury-vapour lamp for 2 hours. They found that 1 mgrm. of this material produced marked resumption of growth, and at this time they believed that irradiation of cholesterol produced vitamin A. Rosenheim and Webster (1926) made the suggestion that the action of irradiated cholesterol on growth was apparently identical with that of ultra-violet light and appeared to consist in mobilising the animal's reserves

of vitamin A. Steenbock and Nelson (1925), however, put forward quite a different explanation, suggesting that vitamin A was not produced by irradiation of cholesterol, but that the growth-promoting effect produced was due to the conferring of antirachitic powers upon it. They put forward the theory that vitamin A was not the true growth-promoting vitamin, since irradiation of a rat which had ceased to grow, without any sign of ophthalmia, promptly reinstated growth, while additional amounts of vitamin A would not elicit such a reaction. Their views received some support from observations of Rosenheim and Drummond (1925), who, having elaborated a delicate colour reaction for vitamin A, found that cholesterol did not give this colour reaction. Hess (1925) confirmed this work, finding cholesterol and phyto-sterol antirachitic after exposure to ultra-violet light, and Rosenheim and Webster (1926), working simultaneously, found that the stability of activated cholesterol was greater if the irradiation took place in an atmosphere of nitrogen than if it took place in air. Attempts were made to separate an active fraction from irradiated cholesterol; an amorphous substance was obtained in this way which conferred partial protection from rickets on rats receiving 0.01 mgrm. a day.

Hess, Weinstock, and Sherman (1926), on further investigation, found healing properties not only in dried milk, but in human milk when the mother had been exposed to irradiation, in cholesterol and certain vegetables, and also in animal's skin. Feeding young rats on human or calf's skin which had been exposed to ultra-violet light protected them regularly from developing rickets on a deficient diet; Hess and Weinstock therefore formulated the suggestion that solar rays might produce an antirachitic effect by activating cholesterol present in an animal's skin.

The idea of the antirachitic property of skin was further supported by the work of Falkenheim and, later, by Rekling (1927), who showed that irradiation of rats, after shaving the skin, raised the inorganic phosphorus of the blood to a much greater extent than when the fur was left intact.

From these facts Aidin (1927-1928) formulated the conception that the skin acts as a gland of internal secretion, whose function is active from infancy during the period of growth up to the time of puberty. So far, the investigations of all the workers on the source of the antirachitic vitamin had led them to the conclusion that cholesterol was the substance which was rendered antirachitic by the action of ultra-violet light.

Special confirmation of this view seemed to be received from the reports in 1925 of Hess and Weinstock, and of Steenbock and his co-workers, that the purest cholesterol obtainable when treated with ultra-violet rays developed marked antirachitic potency.

Rosenheim and Webster (1927) now took up the investigation with the object of isolating, or at least concentrating, the active fraction of irradiated cholesterol. They found that recrystallisation from alcohol in the presence of air rendered cholesterol incapable of being activated, as did also purification by converting it into the dibromide, and they further succeeded in obtaining from irradiated cholesterol by the aid of digitonin, a substance which was intensely active. They were unable, however, to convert more than about 0.01 per cent. of the cholesterol into this active substance. The effect of irradiation on various cholesterol derivatives and sterols was also investigated, and from the fact that while cholesterol acetate and palmitate and ergosterol were rendered active, while coprosterol and amyrol, members of the group of excretory sterols, remained inactive, the conclusion was reached that unless both the unsaturated linkage and the hydroxyl group of the cholesterol molecule were intact activation was impossible.

The possibility that the absorption spectrum of cholesterol might be due to a small amount of an impurity had been suggested by Schlutz, Zeigler, and Morse (1927) in their study of the absorption spectra of cholesterol. A specimen of purified cholesterol, prepared

by Hess and Windaus (1927) by way of the dibromide, was now found no longer to show the characteristic spectrum of ordinary "pure" cholesterol, and Heilbron, Kamm, and Morton (1927) made the suggestion as the result of their spectrum analysis, that the activation of ordinary cholesterol might be due to the presence in it of traces of an unknown substance.

With these results, both chemical and optical, in mind, Rosenheim and Webster (1927), in collaboration with Windaus, took up the study of ergosterol. Its discoverer, Tanret (1889), had already (1910) studied the oxidation of ergosterol under the influence of light and air, and it was known to have two other properties in common with the activable substance in cholesterol, namely, its destruction by bromine, and the formation of an insoluble digitonide. In view of the work of Heilbron, Kamm, and Morton (1927), and of Pohl (1926), the absorption spectrum of ergosterol was compared with that of a specimen of ordinary cholesterol. It was found that ergosterol showed a very pronounced absorption in the ultra-violet region, the characteristic band of absorption being like that of cholesterol, at $280\mu\mu$, but of much greater intensity, 1500 to 2000 times as strong. As the result of irradiation this absorption band disappeared, just as in the case of cholesterol; at the same time the product lost its property of being precipitated by digitonin. If the provitamin were ergosterol, the amount present in ordinary cholesterol would be about 1 part in 2000, and this assumption was fully confirmed by biological tests on rats. Tests indicated that the minimum curative dose for rickets of irradiated ergosterol was less than $\frac{1}{1000}$ mgrm. per day. From these results the conclusion was reached that the naturally occurring parent substance of vitamin D was either ergosterol itself or a highly unsaturated sterol of similar constitution.

It should be mentioned here that Bills and co-workers (1928) find that even strictly purified cholesterol does bring about slight healing in rickets when re-irradiated and fed in quantities thirty times as great as the original "pure" cholesterol. They believe this effect to be due not to a small amount of persisting impurity but to the very weak activability of cholesterol itself.

XXIX. SOURCE OF VITAMIN D.

(1) **NATURAL SOURCES.**—The source *par excellence* of vitamin D is cod-liver oil and other natural animal fats, where it accompanies vitamin A in the non-saponifiable fraction of complex unsaturated alcohols.

Ergosterol, now recognised as the parent substance of vitamin D, appears to be the most widely distributed of all sterols, occurring not only in the animal kingdom but the vegetable.

Its presence in the fungus ergot of rye was discovered by Tanret in 1890, and Mellanby and co-workers (1929) have recently shown that ergot has a powerful calcifying action on the bones of rachitic dogs. All samples of ergot tested, whether of Russian or Spanish origin, were antirachitic, some samples roughly possessing one-eighth to one-quarter the calcifying activity of cod-liver oil.

It can be extracted from yeast by a method described by Windaus and Grosskopf (1923). According to Heiduschka and Lindner (1929) the ergosterol content of different yeasts differs widely, and, for the same yeast, variations in the nutrient solution cause great variations in the ergosterol content (0.34 to 1.0 per cent.).

More recently (1929) Sumi claims to have isolated ergosterol ($C_{27}H_{42}O$) from the Japanese edible mushroom, *Cortinellus Shiitake*. The substance isolated agrees closely with Tanret's constants, and solutions radiated for 30 min. with the mercury arc have been shown to have antirachitic powers in rats. Dosing a rat with non-irradiated ergosterol, and then irradiating the rat, caused healing. (Mellanby and co-workers (1929), on the other hand, have found no calcifying action with edible mushrooms.)

Raw coffee has recently been indicated by Schwarz and Sieke (1930) as a source of highly active ergosterol.

Ergosterol occurs in close connection with cholesterol ($C_{27}H_{46}O$), which requires vigorous purification before it can be freed from ergosterol.

The samples of cholesterol from cod-liver oil, brain, spinal cord, gall-stones, skin, and blood, tested by most workers, have been found to contain ergosterol in small amounts. Recently, however, Koch, Koch and Ragin (1929) claim to have purified a commercial sample obtained from the spinal cord, so that it appeared to be free from ergosterol.

It has been found also in the eggs, brain, liver, spleen, and ovaries of lower animals, and in the blood of both animals and human beings.

The sterols extracted from red blood corpuscles have been shown by Euler, Euler and Rydbom (1928), to acquire a certain antirachitic activity on irradiation. They consider that ergosterol is present in the corpuscle sterols to the extent of about 1 per cent.

Mouriquand and Leulier (1929) have found that a mixture of the sterols of the snail, *Helix pomatia*, which mixture they call "hélistérine," acquires antirachitic properties on irradiation. In vegetable oils the unsaponifiable fraction has been found to contain ergosterol in close association with phytosterol.

(2) **SYNTHESIS OF VITAMIN D.**—Until recently it was believed that the synthesis of vitamin D in the plant world took place in the same way as that of the other vitamins, and that the ultimate source of vitamin D in cod-liver oil was the green algæ and diatoms which inhabit the ocean. Jameson, Drummond, and Coward showed, in 1922, that the marine diatom, *Nitzschia closterium*, was a rich source of vitamin A, but Leigh Clare (1927) has thrown doubt upon this theory of the origin of vitamin D by showing that *Nitzschia closterium*, grown with conditions of maximum insolation, has no antirachitic power. She suggests, however, that the plankton and the smaller fish might conceivably be exposed to sufficient radiant energy of the necessary wave-length for synthesis of vitamin D. Wejdling (1928) has attempted to obtain evidence for the formation of vitamin D in the food of the cod. He found that the algæ which are found near the surface of the sea, and whose covering consists almost entirely of silica, transmit even the short ultra-violet rays, and that 2 per cent. of these algæ added to the food of rats on an otherwise rachitic diet induced rapid healing of the rickets. Their antirachitic properties could be still further increased by moderate ultra-violet irradiation. The spontaneous antirachitic action of algæ of the Confervæ family, which had been collected in dark spots, protected from the light, has received a somewhat different explanation from Lesné and Clément (1929).

Since these algæ have received no solar radiation, Lesné and Clément suggest that the production of the antirachitic factor, while usually effected under the influence of light, may not belong exclusively to light radiations, but may, in different circumstances, be otherwise effected.

Bills (1927), on the other hand, has ascertained that the principal food (*caplin*) of the Newfoundland codfish apparently does not contain enough vitamin D to account for the quantity accumulated by the cod during its midsummer period of fattening.

The vitamin D of catfish oil was not increased by irradiating the fish, nor was it decreased by keeping the fish for six months in the dark on a vitamin deficient diet. Later experiments have led to the belief that the antirachitic vitamin is not, as formerly believed, entirely dependent on light energy. Voltz and Kirsch (1927) (1928) consider that, to a certain degree, vitamin D is formed in the metabolism of plants without the influence of ultra-violet rays. They found that grass (*Lolium perenne*) which had grown and become green under common window glass, as well as grass which had germinated in a perfectly dark room, and was etiolated, when fed to rachitic rats on McCollum's diet 3143, brought about healing processes as demonstrated by the X-ray.

Schittenhelm and Eisler (1928) further found vitamin D present in the first sproutings of barley, which had been kept in dark rooms, so that all question of an action of light had been excluded, and as yet they have been unable to demonstrate ergosterol in the sprouts. Apparently, then, in this case, there is no preliminary stage (provitamin) of the antirachitic substance, and vitamin D can originate in the process of germination.

Bills suggests, on the basis of these observations and his own on the occurrence of vitamin D in fish oils, that vitamin D may be actually elaborated in the body of some marine animals without the intervention of light. Maignon (1927) has brought forward the interesting suggestion that vitamin D is synthesised from its fat-soluble companion, vitamin A, in the liver, and that it is the derangement of hepatic function that accounts for the deficiency of vitamin D in rickety children. He supports his statement by the results of irradiation of animals, for instance, cattle, whereby the antirachitic power of the milk is greatly increased; Maignon considers this fact to prove that vitamin D can be synthesised in the body. The synthetic formation of vitamin A and D in the body was also suggested by Euler and Rydbom in 1926. They obtained from yeast and muscle a cozymaze identical with that known to be present in the erythrocytes, and found that it stimulated the growth of rats on A- and D-deficient diets.

(3) **CHEMICAL NATURE OF THE PRO-VITAMIN D.**—Once the fact was established that cholesterol could be activated to become antirachitic by irradiation, investigation of its various fractions and their activability was undertaken.

(a) **Cholesterol Fractions and Vitamin D.**—Shear and Kramer (1926) found that irradiation of cholesterol in air for one hour at 18 inches resulted in about 5 per cent. of a yellow oil, provisionally called "V. V. oil of cholesterol," which remained in the mother liquor on recrystallisation of the cholesterol from acetone and methyl alcohol. About 40 per cent. of this crude oil was not precipitable by digitonin, and this fraction was antirachitic, while the precipitable fraction was inactive. The curative dose of the active fraction, according to Kramer, Shelling, and Shear (1926, 1927), was equivalent to 2 c.c. of cod-liver oil. This potent cholesterol-free concentrate gave a decidedly positive reaction with Shear's aniline hydrochloric acid-reagent.

(b) **Cholesterol Derivatives and Vitamin D.**—Various compounds and oxidation products of cholesterol have been examined for antirachitic activity. Bills and Macdonald (1927) subjected to irradiation 4 esters and 14 ethers of cholesterol, all inactive in preventing rickets. Of the esters, cholesterol acetate, isobutyrate, and benzoate became markedly antirachitic, but the cinnamate remained inactive. Diergosteryl phosphate is stated by Euler, Euler and Rydbom (1928) to be as active when irradiated as irradiated ergosterol itself.

Certain oxidation products of cholesterol, α - and β -cholesterol oxides, cholestatriol and hydrox-cholesterol were found by Schultz, Ziegler, and Morse (1927) to remain inactive after irradiation, indicating that with only slight changes in the cholesterol molecule the activating factor had been destroyed. β - and γ -sitosterol, prepared from corn-oil after crystallisation by Hess and Anderson (1927), were not susceptible to activation, while α -sitosterol, the fraction which had not been brominated, was found to be strongly antirachitic.

Another series of derivatives have been investigated by Heilbron and Sexton (1929) in the attempt to prove that ergosterol is present in all phytosterols. Dihydro-sitosterol or γ -sitostanol is the hydrogenated derivative of γ -sitosterol—a sterol found in corn oil. This substance shows many similarities of physical properties to alloergostanol, the completely hydrogenated form of ergosterol. Heilbron and Sexton suggest that ergosterol may be formed by the oxidation of sitosterol (by loss of hydrogen), this taking place concurrently with the reduction of part of the sitosterol to sitostanol.

Irradiated and non-irradiated ergosterol peroxide has been found by Heubner and Holtz

(1929) to have no antirachitic activity, and irradiated chaulmoogric acid-allyl ester was also found inactive by Hottinger (1929).

All these facts were interpreted and reconciled by Rosenheim and Webster's explanation (1928) that the potent factor, ergosterol, occurring as an impurity with cholesterol, was absent in the non-activable compounds, and that the receptive substance was a sterol of an unsaturated and labile type, possessing three double bonds and a hydroxyl radical, of which ergosterol was the only known representative.

(c) **Specificity of Ergosterol as the pro-Vitamin D.**—The question was raised whether the photo-chemical change connected with vitamin D formation was specific for ergosterol, or whether other substances with three or more double bonds, but not possessing the sterol ring structure, would also yield the vitamin on irradiation. Several compounds having such chemical configuration have been investigated by Rosenheim and Webster (1928, 1929), among them the saturated sterols, coprosterol and sitosterol, with one double bond, and stigmasterol and cholesterolene with two double bonds. Other substances tested include the hydrocarbon squalene with six double linkages, sphingosine, phrenosin and iso-ergosterol. None of the substances showed antirachitic activity after irradiation. Ergosterol peroxide also, irradiated or otherwise, has been shown by Heubner and Holtz (1929) to possess no antirachitic properties.

Further confirmation of the view that only a molecular structure, such as that possessed by ergosterol, enables a sterol to be photo-chemically converted into vitamin D has been received from experiments of Bills, Honeywell, and McNair (1928), and other workers. Bills and co-workers have stated, from a comparison of destruction rates by potassium permanganate, that the activable, ergosterol-like impurity in cholesterol actually is ergosterol. At the same time these workers observed that cholesterol specially treated with charcoal or bromine for the removal of ergosterol was activable by ultra-violet rays. They concluded that the activability was due either to cholesterol itself or to a hitherto undiscovered impurity which persists after three purifications with bromine. This observation, as will be seen below, has been followed up by Koch and co-workers, leading to somewhat similar conclusions.

Whether the antirachitic vitamin in cod-liver oil is actually identical with irradiated ergosterol is not absolutely certain, but Adam (1927, 1929) has provided strong evidence of close similarity in properties between irradiated ergosterol and the fraction of cod-liver oil extracted with acid alcohol. The product of extraction has a very similar spectrum to that of irradiated ergosterol, and protects rats from rickets. Neutralisation of this acid alcohol extract and subsequent treatment with digitonin in no way alters the spectrum—an observation which falls into line with that of Windaus, namely, that irradiated ergosterol is not precipitated by digitonin.

Although ergosterol is a substance of very labile character, King, Webster, and Rosenheim (1929) have found it undegenerated and capable of activation by irradiation in a mummified brain 1400 years old. The brain was of Coptic origin, dating from about A.D. 500, taken from a body found in tombs in Antinoe in Upper Egypt, and yielded 12 per cent. of cholesterol esters. The recrystallised esters gave positive colour reactions for ergosterol with trichloroacetic acid and with bromine in chloroform solution, and a 5 per cent. ether solution of the esters showed the absorption spectrum of ergosterol. After irradiation the esters showed, when tested on rats, an antirachitic activity equal to that of an equivalent amount of cholesterol from fresh brain. It is possible that the ergosterol remained unchanged, owing to the protective action of the cholesterol with which it was associated. Bills, Honeywell, and McNair (1928), referring to a sample of highly activable gall-stone cholesterol, 16 years old, suggest that cholesterol may function as an anti-oxidant for its admixed ergosterol, just as some sterol-like substance serves as a natural anti-oxidant in crude rubber. Zymosterol,

the dextro-rotatory sterol isolated from yeast, has been closely examined by Smedley Maclean (1928) and others. Both ergosterol and zymosterol are precipitated by digitonin and contain at least three ethenoid linkages. By a prolonged series of recrystallisations, Penau and Tanret (1929) have prepared the sterol in a highly purified form. They confirm the probable existence of three ethenoid linkages, but conclude that the formula for the sterol is $C_{27}H_{42}O_2 \cdot H_2O$, the substance differing in chemical structure as well as optical activity from ergosterol.

Fabre and Simonnet (1929) report that this purified substance after irradiation possesses some antirachitic properties, although to produce the same degree of healing in rachitic rats, at least one hundred times as much of the sterol is required as of irradiated ergosterol. It is possible, however, that this slight curative effect may be due to the presence of traces of ergosterol which, despite the prolonged series of recrystallisations, may still exist in the purified preparations.

Another explanation, however, is suggested by the recent work of Koch, Koch and Ragins (1929), and Koch, Koch and Lemon (1929). They found that a commercial preparation of cholesterol, containing ergosterol, even when highly purified by several methods, was still capable of being rendered antirachitically potent by irradiation. The potency was reduced by these purifications, which included repeated washings, recrystallisations, bromine treatment, and boiling with potassium permanganate, to 1/70th to 1/30th of its original potency, but the purified products could be again rendered at least twenty-five times more activable if heated under proper conditions slightly above the melting-point of cholesterol. Koch and co-workers interpreted these results to show that the specificity of ergosterol as the parent substance of vitamin D was not absolute, as had hitherto been believed, and undertook absorption spectra studies in order to confirm their interpretation.

The purified products, though activable, did not show the absorption bands corresponding to ergosterol, nor any general absorption in the ultra-violet region. When the activability of the purified products was increased by special methods of heating, they showed no bands whatever, though a strong general absorption in the ultra-violet region was observed, parallel with their increased activability. From this evidence, Koch and co-workers conclude that the provitamin activity of the sample of cholesterol used was due not only to the presence of ergosterol, but to that of another substance or substances, probably in part cholesterol itself, and in part a modified cholesterol of unknown chemical character. While unable definitely to state the nature of the chemical change produced by the melting and heating process, they suggest, on the basis of studies by Diels and Abderhalden (1906), Diels and Linn (1908), Windaus and others (1912), that substances such as β -cholesterol, cholestanon, dihydro-cholesterol, allocholesterol, etc., may be produced, and that the increased activability may be due to one or more of these.

(d) **The Mechanism of Formation of Vitamin D.**—The actual formation of vitamin D is not yet entirely understood, though the accepted opinion seems to be that the activation consists of a photo-molecular change (an intra-molecular rearrangement) rather than a photo-oxidation. The acetate and benzoate can be activated to the same degree as ergosterol itself, and therefore the hydroxyl does not appear to be concerned in the change.

Bills and Brickwedde (1928) have found activation by ultra-violet light of a sample of cholesterol containing 0.12 per cent. of ergosterol to occur to a considerable extent at liquid oxygen temperature ($-183^\circ C.$). Oxidations and reactions in general, involving two or more different molecules, are repressed at such low temperatures. They suggest that the light absorbed by ergosterol, whose spectral bands disappear with time and after prolonged irradiation, is used in this photo-chemical change to make vitamin D.

A suggestion that the products of irradiation of ergosterol are of a ketonic nature comes

from Rosenheim and Adam (1929), basing their theory on an examination of a film of ergosterol of monomolecular thickness. Bills and Cox (1929) compare the isomerism of ergosterol resulting from treatment with hydrochloric acid, hydrobromic acid, etc., to the process which occurs when activation by ultra-violet rays takes place, and suggest that the same double bond is involved.

Rosenheim and Webster (1927) have shown that the maximum potency is developed after 30 minutes' irradiation, using 0.1 per cent. solutions of pure ergosterol in ether or alcohol enclosed in quartz tubes filled with nitrogen, and that the antirachitic activity of the reaction products does not increase as the ergosterol decreases, but remains constant up to 4 hours' irradiation. It is suggested that after a short initial period the vitamin is formed and destroyed at the same rate, so that the activity remains constant until the available supply of ergosterol is exhausted.

A comparison of the absorption curves characteristic of iso-ergosterol, which is an isomeric form of ordinary ergosterol prepared from it by a chemical method, has led van Wyk and Reerink (1928) to formulate a hypothesis of the mechanism of the photo-chemical change. They suggest that ergosterol has two types of absorption bands connected with different parts of the molecule. By irradiation such that vitamin D is formed, the first system ($\lambda_1 = 293_5$, $\lambda_2 = 281_3$, $\lambda_3 = 270_9$) makes way for the characteristic absorption band of iso-ergosterol, which would mean that the corresponding part of the molecule undergoes the same change in constitution by irradiation as by the transformation of ergosterol into iso-ergosterol by the chemical method of Reindel. Another part of the molecule obviously does not change its constitution. Like vitamin D, the iso-ergosterol remains unchanged by irradiation with ultra-violet light of wave-length longer than $270 \mu\mu$, and is destroyed by light of wave-length of about $250 \mu\mu$, as is shown by the disappearance of the absorption bands. By using the two methods of irradiation ("short wave" and "long wave"), and removing the unchanged ergosterol, Reerink and van Wyk (1929) claim to have prepared pure vitamin D in crystalline form. The crystals are said to melt below 0°C . The absorption spectrum of this product agreed well with that calculated from the curves obtained by irradiating ergosterol. Its antirachitic potency as judged by feeding tests on children and on rats was greater than any product hitherto obtained, daily doses of 0.00001 mgrm. for 2 weeks sufficing completely to cure rickets in young rats which had been fed on a deficient diet during the preceding 4 weeks.

Windaus (1930) also by various methods of irradiation has recently succeeded in separating the antirachitic from the "hypercalcæmic" or toxic factor in inosterol; the latter is far more resistant to the rays than the former.

Kon (1928), however, regards the assumption that vitamin D is a product of photo-chemical activity as premature. He has measured the light energy absorbed by ergosterol spread in a thin layer on a thermopile, and has found that there is no appreciable absorption of heat energy in its photo-chemical activation. He considers it probable that most of the energy absorbed during the activation of ergosterol is dissipated as heat or used in bringing about the formation of new products other than vitamin D.

It may be noted in passing that Glanzmann (1927) has gone still further in his non-acceptance of the theory of the photo-synthesis of vitamin D and advances a theory of his own for which there seems little substantiation. He considers that the important change that takes place during the irradiation of cholesterol is a polymerisation, which renders the cholesterol chemically less active. In all tissues there is an equilibrium between the antagonistic substances lecithin and cholesterol; if the lecithin were allowed to function without restraint it would promote normal calcification of bone, but the cholesterol antagonises it, unless it is partly inactivated by becoming polymerised under the influence of ultra-violet light. Glanzmann thinks that this hypothesis explains the cure of rickets by light without

any assumption of the synthesis of an antirachitic vitamin, but unfortunately he produces no evidence whatever to show that lecithin itself can promote the healing of rickets.

Mellanby and co-workers (1929) have recently made the interesting statement that natural ergot, which contains ergosterol, has a powerful antirachitic action without previous exposure to ultra-violet light, while Adam has observed that the ultra-violet absorption spectrum of insulin is very similar to that of ergosterol.

Serono and Cruto (1928) have made the suggestion that it is the phosphatides, widespread in vegetable and animal organisms, which have the function of storing up the solar energy which they afterwards use in the formation of vitamin D. They observe that phosphatides have the property of re-emitting ultra-violet rays, and that this property may prove to form the connecting link between vitamin action and irradiation.

(4) **ABSORPTION OF VITAMIN D FROM THE SKIN.**—The majority of workers appear to have come to the conclusion that the skin plays a definite part in the absorption of ultra-violet light and its subsequent action on the precursor of vitamin D.

Hess and Weinstock found in 1925 that pieces of irradiated skin from infants and calves exercised an antirachitic action when eaten by rats. The existence of cholesterol in the epidermis (to the extent of 13 to 24 per cent. in pigs' skin) was shown in 1915 by the researches of Rosenheim and Webster, and it has been suggested by Hess, Weinstock, and Helman (1925) that the protection of animals from rickets might be due to activation of this cholesterol after absorption.

The epidermis is very opaque to a wave-length of about $280\ \mu\mu$, but for ultra-violet light of a slightly greater wave-length it is much less opaque. Hume, Lucas, and Smith (1927), calculating from Hasselbalch's observations (1911), have shown that $1/56$ th of the incident light of a wave-length of 297 penetrates 0.1 mm. and $1/3000$ th penetrates 0.2 mm. of epidermis.

The above workers carried out experiments to test whether irradiated cholesterol, applied to the external surface, could exercise its antirachitic potency through the skin. The skin of young rats and rabbits was depilated of hair. To the depilated area, cholesterol, which had been dissolved in ether, evaporated in a thin layer on a glass slide, and irradiated for 10 minutes at a distance of 16 inches from a mercury-vapour quartz lamp, was applied three times a week, over an area of about 1 square inch. Controls were left untreated, and other animals were irradiated directly. The results showed that, both histologically and in their percentage ash, the bones of the animals which had had cholesterol applied to their skins approached those of the animals irradiated directly, while the controls showed rickets histologically and a considerably lower percentage of ash. These experiments showed that vitamin D in irradiated cholesterol can be absorbed from a small area of undamaged skin in sufficient amount to supply the needs of the animal, and supported the suggestion of Hess (1924) that activation of cholesterol in the skin by sunlight, and its subsequent absorption, is a possible source of the vitamin.

Rekling (1927), however, has described experiments which, he suggests, invalidate the above results. He treated rats on a suitably deficient diet with ultra-violet light, but in such an apparatus that they were prevented from absorbing their own secretions by the mouth. These rats, in spite of the administration of ultra-violet light, developed rickets just as non-irradiated controls did: on the other hand, rats irradiated under the ordinary conditions without the special apparatus were protected from rickets. He suggests, therefore, that cure of rickets is not due to the resorption of cholesterol by the skin, but that the action of light is an indirect one, due to the ingestion of activated secretions, and especially the cholesterol of serum.

(5) **METABOLISM OF ERGOSTEROL.**—Apparently ergosterol and cholesterol have a different

metabolism. When ergosterol is taken by the mouth a considerable portion is retained, whilst cholesterol is again excreted in the bile.

Beumer (1927) has investigated the balance of ergosterol in a suckling child, to which it was given in periods of 4 days (0.1 gram of ergosterol in 10 c.c. of olive oil), and 300 c.c. of skimmed human milk, fed in 10 separate feeds. The entire stools during the different periods were extracted with alcohol, and the alcohol soluble fraction divided into two equal parts. One part was irradiated with ultra-violet light whilst in a quartz tube so as to exclude air. Ergosterol is not precipitated by digitonin after it has been irradiated, so that the amount of ergosterol excreted could be estimated by the difference in the digitonin precipitate of the two samples. 147 mgrms. of ergosterol was recovered from the 400 mgrms. ingested; after 3 days the amount being excreted was so small as to be of no account. The unrecovered ergosterol might either be stored or disintegrated. Beumer suggests the latter as the more likely possibility, since ergosterol is more easily oxidised than cholesterol.

That ergosterol is present in human blood has been shown by Dejust, van Stolk, and Defreuil (1928), by extracting the sterols from a collection of human blood obtained from numerous different samples. The ultra-violet spectrum given by a saturated alcoholic solution of the extracted sterols showed the three characteristic absorption bands typical of a solution of non-irradiated ergosterol.

Euler and co-workers (1928) have also shown that the blood of warm-blooded animals has marked growth-promoting and antirachitic powers when fed to rats on a diet deficient in vitamins A and D. This effect was found not to be due to the iron-containing compounds.

Euler and Rydbom (1928) extracted the sterols from ox blood and found that doses of 0.1 mgrm. allowed growth for some weeks on a diet devoid of vitamins A and D. The same dose of irradiated sterol caused, in about 75 per cent. of rats, a greater increase in growth, which was maintained for 10 weeks and longer. The irradiated sterol prevented rickets in as small a dose as 0.05 mgrm. a day. (The non-irradiated sterol had no antirachitic effect.) Taking the minimum curative dose of irradiated ergosterol as 0.0001 mgrm. in rats they give the value of 2 per cent. for the ergosterol content of the blood sterol. From their results they suggest that a large part of the blood sterol is not in activated form—a suggestion which fits in well with the known effects of direct animal irradiation. Irradiation of preparations of cholesteryl phosphate and phosphite was shown to make them strongly antirachitic. This result was probably due to the effect on similar salts formed from contaminating ergosterol.

These results have been confirmed by recent experiments by Giaume (1929). White rats, which had been subjected to a rachitic diet, were given 1.5 c.c. per 100 grms. of their total food of the blood from a rat previously treated with ultra-violet light. After 3 weeks these animals remained healthy, while controls, which had received blood from a normal adult rat, showed marked signs of rickets. Giaume suggests that the ergosterol contained in the cholesterol of red blood corpuscles becomes activated by irradiation, thus giving antirachitic properties to the blood.

XXX. PHYSICO-CHEMICAL PROPERTIES OF VITAMIN D.

(1) **FACTORS AFFECTING STABILITY.**—The difference of stability of vitamins A and D with respect to various agents forms one of the means of differentiating between the two factors in presence of each other, vitamin D being on the whole decidedly less susceptible to destruction than vitamin A.

(a) **Solubility.**—Vitamin D is soluble, according to Shipley, Kinney, and McCollum (1924), in alcohol, ether, and acetone, but its degree of solubility depends to some extent upon the source from which the extraction is made. In the case of spinach, Brussels sprouts, cabbage, celery, and tomatoes, ether fails to extract vitamin D; while Randoin and Simonnet

(1927) state that, occurring in lucerne, it is incompletely soluble in ethyl acetate and partially boiling water. (Ackerson, Bligh, and Mussehl (1925) have failed to confirm the latter statement.)

(b) **Oxidation.**—Vitamin D resists oxidation in the presence of air more strongly than vitamin A, the antirachitic potency of cod-liver oil not being destroyed after being subjected to the action of oxygen at 100° C. for periods varying from 12 to 28 hours. According to Bills (1925), it is destroyed by oxidation by *n*-butyl nitrite.

Holtz (1927) has shown that the active substance produced by the absorption of ultra-violet rays by ergosterol is readily destroyed by oxidation with bromine or permanganate, and loses its activity as a vitamin when exposed to the air. Kept in paraffin solution, it retains its activity undiminished.

(c) **Heat.**—The antirachitic vitamin in cod-liver oil is not destroyed by being heated for 4 hours in the autoclave, nor for 22 hours at 100° C. in presence of air.

Heuser and Norris (1929) have subjected cod-liver oil to oxidation at the temperature of boiling water by means of carbon dioxide-free, moisture-free air. The results indicate some destruction of vitamin D after 12 hours, material destruction after 24 hours, and complete destruction after 48 hours.

(d) **Precipitation.**—When treated with digitonin, vitamin D forms an insoluble additive compound, and the reaction product is no longer activable.

(e) **Alkalies.**—Saponification, especially if oxidation is avoided, does not destroy vitamin D.

(f) **Acids.**—According to Bills (1925), hydrolysis with 0.5 N. HCl does not destroy vitamin D.

(g) **Bromination.**—Vitamin D is destroyed by treatment with bromide. Cholesterol, stigmasterol, and phytosterol, when converted into dibromide and subsequently reduced, although remaining unchanged as to melting-point and colour reactions, were found by Rosenheim and Webster (1927) to have lost their antirachitic power on irradiation.

(h) **X-Rays.**—X-rays appear to exert a destructive effect. Morrison, Peacock, and Wright (1928) found that large doses (8 pastille), acting on dried ergosterol in air, rendered it incompletely protective against rickets.

Stentstrom, Lohmann, and Hillstrom (1928) have also found no healing in rats receiving a chloroform solution of cholesterol irradiated with X-rays.

According to Sumi (1929), no change occurs in ergosterol or its irradiated product, vitamin D, by irradiation with X-rays for a short time, but when they are submitted to heavy treatment by X-rays there is a diminution of rotatory power, in amount of precipitate by digitonin and in sensibility to colour reaction. Even prolonged irradiation by X-rays failed to activate ergosterol to vitamin D, and ergosterol activated by ultra-violet rays was gradually destroyed, as was most of its activity as vitamin D. The spectrum of ergosterol and activated ergosterol extended farther into the ultra-violet region, and the band near 239 $\mu\mu$ became obvious after X-ray irradiation for 6 to 8 hours.

(2) **FACTORS IN THE STABILITY OF ACTIVATION OF VITAMIN D**—(a) **Time.**—The effect of time varies with the method of preservation. In alcoholic or etheric solution, cholesterol was found by Heilbron, Kamm, and Morton (1927), confirming the results of Schultz, Ziegler, and Morse (1927), to show no absorption bands after standing 4 hours. In the dry state or in aqueous solution, activated cholesterol loses its property after 15 days' exposure to light. In oily solution, on the other hand, it preserves its activity for 8 or even 10 months.

(b) **Length of Time of Irradiation.**—Both in cod-liver oil and cholesterol the antirachitic factor is inactivated by too long irradiation. Adam (1929) suggests that the sterol of cod-liver oil is inactivated by desaturation, while cholesterol, by action on the double bond, is converted into a new combination.

(c) **Solvents.**—Cholesterol can be activated only in solvents transparent to ultra-violet radiation of the wave-lengths covered by the absorption bands. Heilbron, Kamm, and Morton (1927) found no activation of cholesterol in acetone—a fact which had been recorded in 1925 by Hess, Weinstock, and Sherman.

(d) **Wave-Length of Ultra-Violet Light.**—The short ultra-violet rays (beyond $260\mu\mu$) have been found by Rosenheim and Webster to be destructive to vitamin D, and they have suggested that in order to obtain the maximum production of vitamin D the rays shorter than $260\mu\mu$ should be screened off.

(3) **COLOUR TESTS FOR VITAMIN D**—(a) **Shear's Aniline HCl Test.**—Shear (1926) has described a colour reaction in connection with vitamin D which, though it has not been proved an absolute specific test for the vitamin, seems to indicate a close connection between the chromogenic substance formed and the antirachitic factor.

Shear (1926) reported that cholesterol, which had been exposed to ultra-violet radiation from a mercury-vapour quartz lamp, reacted differently from untreated cholesterol towards a reagent consisting of aniline and concentrated hydrochloric acid, the irradiated cholesterol giving a bright red colour.

Rosenheim and Webster (1926) confirmed this observation, and, while not regarding it as specific for vitamin D, considered it to show direct chemical evidence of a change in cholesterol following irradiation in air for short periods. Shear found that the unsaponifiable fraction of cod-liver oil gave a more intense red than did the oil itself, and that the intensity was not diminished when the vitamin A in the oil was destroyed by oxidation at 100°C ., its destruction being evidenced by the fact that the colour tests for vitamin A of Rosenheim and Drummond were negative. Other inert oils, such as cotton-seed oil, linseed oil, olive oil, and coco-nut oil, which did not give the red colour before irradiation, reacted strongly after exposure to ultra-violet light.

Sexton (1928) has confirmed these findings, and has also found that the reaction was given by irradiated ergosterol but not by the unirradiated sterol. Ketones proved most sensitive to the test, but the singly unsaturated ketones alone gave the red colour to any marked degree. These results are in harmony with the view that vitamin D is possibly ketonic in character. It should be noted, however, that Euler, Myrbach, and Karlson (1926) deny that the products arising from irradiation of cholesterol, betulin, cod-liver oil, and pea-nut oil have any relation to the vitamin properties of the oil, although they are responsible for the "colour" reaction.

(b) **The Phosphorus Pentoxide Test.**—Stoeltzner (1928) has described a reaction of vigantol oil—a solution in olive oil of the antirachitic vitamin—with phosphorus pentoxide, giving a reddish-brown colour which darkens and finally becomes black, which he suggests may be characteristic of the antirachitic vitamin. The reaction is not given by ordinary olive oil, nor by a solution of non-irradiated cholesterol in olive oil, but is given by liver oils, and by a variety of other substances such as proteins, carbohydrates, glycerol, and organic acids.

Christensen (1925) has suggested that it may be specific for vitamins and hormones, since he has found it positive for preparations of testicles, ovaries, and insulin; but Forschner and Hottinger (1929) have proved it to be not specific for these substances.

(c) **Rosenheim's (1929) Chloral Hydrate Reaction.**—A deep carmine-red solution, changing within a minute to green, and then to deep blue (with an absorption band at $500\mu\mu$), is produced when 1 mgrm. of crystals of ergosterol is added to 0.59 mgrm. of chloral hydrate and liquefied by warming in a water-bath. The colour is discharged by the addition of water or alcohol.

(d) **The Trichloroacetic Acid Reaction.**—Rosenheim (1929) has also described a reaction

between a solution of ergosterol in chloroform and an aqueous solution (9 parts of acid to 1 part of water) of trichloroacetic acid, with the production of a red solution, with an absorption band at $500\mu\mu$, changing to clear blue, without the intermediate green.

(e) **Fuchsin Sulphuric Acid Test.**—Blunt and Cowan (1929) state that irradiated ergosterol gives a violet colour with a fuchsin-sulphuric acid reagent while the non-irradiated does not.

(f) **Acetic Anhydride Test (Meesemaeker (1930)).**—A new colour reaction which is claimed to be specific for ergosterol and to differentiate it from irradiated ergosterol is described by Meesemaeker (1930). He finds that the addition of acetic anhydride followed by anhydrous zinc chloride to a solution of ergosterol in chloroform produces a rose colour changing to a stable green. One-hundredth of a mgrm. of ergosterol in 1 c.c. of chloroform plus 0.5 c.c. acetic anhydride and a small amount of ZnCl_2 gives a definite rose colour. The addition of zinc chloride or phosphorus pentoxide to a chloroform solution of ergosterol (without acetic anhydride) gives a rose colour with a freshly prepared sample of ergosterol which has been kept in the dark. With ergosterol which has been exposed to daylight or irradiated with ultra-violet light, a green colour is produced, the intensity of which appears to be proportional to the time of irradiation.

(4) **OTHER CHEMICAL TESTS**—(a) **Precipitation by Digitonin.**—Ergosterol, like cholesterol, is practically completely precipitated by digitonin, and the filtrate contains only traces of material. Irradiated ergosterol is not completely precipitated, and the filtrate gives, on evaporation, a transparent glassy hard solid, with strong antirachitic potency much more soluble in alcohol than ergosterol, and having an entirely different melting-point and different absorption of light.

Webster and Bourdillon (1928) consider that this substance contains vitamin D if it is not vitamin D itself.

(b) **Reduction of Silver Oxide.**—Irradiated ergosterol will, according to Blunt and Cowan (1929), reduce ammoniacal silver oxide to a stable colloidal silver solution, while the non-irradiated will not.

(5) **SPECTROSCOPIC NATURE OF PRO-VITAMIN D.**—The investigation of the pro-vitamin has been facilitated by the introduction of spectroscopic tests. Active cholesterol gives a characteristic absorption spectrum in the ultra-violet region, and Rosenheim and Webster (1927) have found ergosterol to possess the same spectrum, the intensity of the absorption, however, being enormously increased. The three characteristic bands occur at $293.5\mu\mu$, $281.5\mu\mu$, and $217\mu\mu$. When ergosterol is irradiated, the intensity of the absorption bands diminishes gradually, a considerable decrease being detectable even after irradiation lasting only for 10 minutes. A new band at $247\mu\mu$ then appears, which Heilbron (1927) has suggested is characteristic of vitamin D itself. On prolonged irradiation the absorption band at $247\mu\mu$ disappears, and at the same time the antirachitic potency decreases.

A method of detecting the presence of ergosterol in certain substances has thus been developed from its spectroscopic relationships. Heilbron, Kamm, and Morton (1927) have tested a number of oils and oil extracts in this way, using kapok-seed oil as a solvent (a transparent sample in a film about 0.1 mm. thick was found to transmit ultra-violet rays down to a wave-length of $240\mu\mu$). The three characteristic bands of ergosterol were detectable with certainty in a 0.2 per cent. solution of ergosterol. Yeast fat showed the characteristic absorption bands most strongly, and cotton-seed oil was also shown to be a rich source of ergosterol. With groundnut oil, some samples gave a positive and some a negative result. Heilbron and co-workers emphasise the importance in these studies of the Grotthus-Draper law of photo-chemistry, which states that only the rays absorbed are effective in producing chemical change. They have found that selective absorption is shown only in cholesterol derivatives containing at least two unsaturated linkages in the molecule. The hydrocarbon

cholesterilene, $C_{27}H_{44}$, obtained from cholesterol by loss of one molecule of water, resembles the provitamin in showing three absorption bands, each shifted nearer the red end of the spectrum. This suggests that two of the three unsaturated bonds in ergosterol occupy the same positions in the molecule as the two double bonds in cholesterilene.

Webster and Bourdillon (1929) have attempted to produce more concentrated preparations of vitamin D by using selected portions of the ultra-violet spectrum in the irradiation of ergosterol. During the early stages of irradiation an increase in absorption over a range of wave-lengths was absorbed. Delaplace and Rebière (1929) found this increase, occurring during the first 6 minutes, especially marked for the wave-lengths $263.5 \mu\mu$ and $289.4 \mu\mu$.

They also found that the ergosterol acquired its maximum biological action after 7 to 10 minutes' irradiation, whereas it was necessary to irradiate for 40 minutes before the absorption band between $289.4 \mu\mu$ and $237.8 \mu\mu$, attributed to vitamin D, became well marked. They have suggested, therefore, that absorption at $290 \mu\mu$ is characteristic of vitamin D rather than the band at $247 \mu\mu$ usually attributed to it. Since ergosterol shows absorption between $260 \mu\mu$ and $290 \mu\mu$, while vitamin D shows it chiefly between $230 \mu\mu$ and $260 \mu\mu$, it was hoped to prevent the destruction of the latter during irradiation by cutting off light of shorter wave-length than $265 \mu\mu$. The light filter used was an alcoholic solution of cobalt chloride. No increase in the antirachitic activity of the product was produced, however, by the use of filtered light. Irradiations were also carried out at a number of different temperatures, using unfiltered light. Between -18°C. and $+77.8^\circ \text{C.}$, products of approximately equal anti rachitic activity were obtained, while those obtained by irradiation at -195°C. and -183°C. were definitely less active. The relatively low temperature coefficient of both the formation and destruction of vitamin D suggests that both reactions are photo-chemical in nature (see also p. 129, Bills and Brickwedde).

It is believed by van Stolk, Dureuil, and Heudebert (1928) that the final destruction of vitamin D in the reaction—ergosterol (u.v.) \rightarrow vitamin D \rightarrow product of decomposition—is not due to injurious radiations from the mercury lamp, but to an oxidation which is not entirely eliminated, but considerably lessened, by performing the experiment in an atmosphere of nitrogen.

Further studies of the action of ultra-violet radiation on ergosterol led Bourdillon (1929), Webster and co-workers to conclude that three substances (or groups of substances) are produced in succession, of which the first shows an absorption band roughly similar to that of ergosterol (maximum $280 \mu\mu$), but more than twice as intense. The second product, which is formed by further radiation of the first, shows a strong absorption band with maximum at $240 \mu\mu$, and has no antirachitic activity. The third substance is formed by further radiation of the second, and shows neither antirachitic activity nor marked absorption. In these experiments the solutions were stirred during irradiation, this serving to increase the yield of vitamin D. Under the conditions employed, maximum absorption was obtained at $280 \mu\mu$ after irradiation for 3 minutes with a 0.005 per cent. alcoholic solution of ergosterol, and after 30 minutes with a 0.1 per cent. solution. The unchanged ergosterol was removed by precipitation with digitonin, evaporation of the filtrate to dryness *in vacuo*, and extraction of the residue with ether (which dissolves the products of irradiation, but not ergosterol-digitonide or digitonin). Throughout the process, care was taken to avoid oxidation as far as possible. Since the first absorption band was accompanied by great antirachitic activity, they argued that vitamin D was in all probability responsible for its formation. Later experiments (1930) by Askew, Bourdillon, and co-workers, however, have led them to state that this inference was not correct. By using light filters, preparations have been obtained showing very high antirachitic power, but relatively low absorption at $280 \mu\mu$. Further, by irradiating ergosterol with radiation only of wave-lengths longer than $280 \mu\mu$ (through a filter of xylene), removing the unchanged ergosterol and re-radiating with short wave-lengths only (through filters of chlorine and bromine), there has been obtained during the second irradiation a considerable rise in absorp-

tion at $280\mu\mu$ simultaneously with a destruction of antirachitic activity. Thus it is not at the moment possible to state what is the true absorption band of vitamin D. From this phenomenon of continual formation of vitamin D, and its further change into inactive material, Jendrassik and Kemenyffi (1928) have formulated a fractionation method for securing a preparation with a potency five times as high as that obtained by simple irradiation of ergosterol. By treatment with ethyl alcohol, they were always able to recover successive fractions of unchanged ergosterol. They have also described a reaction of iodine with ergosterol which is useful in distinguishing between ergosterol and its irradiation products. Evidence obtained by this reaction is in accord with the supposed existence of two different irradiation products.

(6) QUANTITATIVE ESTIMATION OF THE PHOTO-CHEMICAL ACTIVATION OF PRO-VITAMIN D.

—Rosenheim and Webster (1927) have shown that the limiting prophylactic and curative dose of irradiated ergosterol for rats is from $1/10,000$ to $1/20,000$ mgrm. per day, and Coward's experiments (1928) have proved that a calcifying effect can be demonstrated by means of the "line test" with a daily dose of $1/100,000$ mgrm. By irradiating for varying periods 0.1 per cent. solutions of ergosterol in ether or alcohol with the mercury-vapour lamp 20 cm. distant, Fosbinder, Daniels, and Steenbock (1928) have also estimated the quantity of antirachitic material involved in the activation of a receptive sterol substance through a careful photo-chemical measurement of the amount of light of definite wave-length required. Their calculation indicates that about 20 billionths of a gram, or, assuming the Einstein photo-chemical relation, 3.2×10 molecules of vitamin D, is sufficient to produce calcium deposition in a rachitic rat, a quantity so small as to defy any chemical test.

Another method of estimation is that of measuring the quantity of radiant energy necessary to form a requisite amount of vitamin D. Kon, Daniels, and Steenbock (1928) have found this quantity to be constant over a wide range of radiations, 700 to 1000 ergs being necessary for the 256, 265, 280, and $293\mu\mu$ lines. The quantum efficiency was independent of the state in which ergosterol was irradiated, the results being the same for irradiation of the solid or of solutions in alcohol of varying concentration.

(7) THE ANTIRACHITIC WAVE-LENGTH.—Experiments to determine which of the ultra-violet rays exert an antirachitic power have resulted in the discovery that the effective rays are those occupying the middle portion of the ultra-violet spectrum ($260\mu\mu$ to $300\mu\mu$).

Hess and Weinstock (1927) tested the lines in the mercury-vapour spectrum from $313\mu\mu$ to $302\mu\mu$ by feeding irradiated cholesterol in equal amounts to rachitic rats, and found that the longest wave-length capable of protecting the animals was in the neighbourhood of $302\mu\mu$.

Further experiments on two bands of shorter wave-length—less than $290\mu\mu$, and between $290\mu\mu$ and $313\mu\mu$, revealed the fact that those shorter than $290\mu\mu$ were more powerfully antirachitic than the most potent region of the solar spectrum.

This observation was further confirmed by Hess and Anderson (1927), by Maughan (1928), and by Griffith and Spence (1928), who examined three separate specimens of inactive ergosterol exposed in thin layers upon glass plates to different regions of the spectrum. The ergosterol on the first plate, A, was thus exposed to wave-lengths greater than $313\mu\mu$; that on the second plate, B, to light between $313\mu\mu$ and $265\mu\mu$; and that on the third plate, C, to wave-lengths shorter than $265\mu\mu$. After irradiation, the samples of ergosterol were dissolved off the glass plates and fed (0.01 mgrm. per day) to groups of rats on an otherwise rachitic diet. It was found that rats receiving the long wave irradiated ergosterol A developed rickets, while those rats on the medium and short wave irradiated ergosterols B and C were free from rickets. It appears, therefore, that the shorter waves of artificial radiation are of biological value, and that the antirachitic rays of sunlight are restricted to the small band of rays at the extreme end of its spectrum.

A warning against a too strict interpretation of the results obtained by employing light filters is issued by Bunker and Harris (1930). They point out that when adequate precautions are taken, as in the investigations of Sonne and Rekling (1927), to secure a constant product of time and intensity, the limits for antirachitic activity seem to be decidedly under the generally accepted limits. In their own experiments, carried out with strict precautions as to rachitogenic diet, diagnosis of rickets, and special sets of light filters, they have obtained many anomalous results, and they have come to the conclusion that there is still a possibility that the future will show a much wider interpretation of the "initial band" of rickets therapy.

The antirachitic value of sunlight, moreover, varies at different times of the year and at different latitudes. At the latitude of Toronto, for example, according to the observations of Brown and Tisdall (1928), the ultra-violet rays constitute only about 1 per cent. of the total solar radiation. Since the wave-length $290\text{ }\mu\mu$ is the effective one, it is obvious that for a great part of the year the antirachitic band is still further restricted; the more so as it is just this extreme region of the solar spectrum that is most readily absorbed by smoke and moisture in the atmosphere.

The results of experiments in Toronto showed that even winter sunshine had slight antirachitic value, but from the end of February onwards this value increased sharply, corresponding with an increase in the shorter rays of the solar spectrum. It is worthy of note that the biological test bears very little relationship to meteorological records of sunshine during the corresponding periods, owing to the ease with which the effective rays are absorbed. A second series of experiments designed to test the relative values of direct sunshine and of "sky-shine" demonstrated that the scattered rays were from a half to two-thirds as effective as the direct sunshine, a point of practical importance as indicating a means of applying an efficient dose of ultra-violet rays in cases where radiant heat is undesirable.

The relationship between the antirachitic effect of sunshine on the altitude of the sun was also investigated by Tisdall and Brown, following the observations of Fabry and Buisson (1921) that the intensity of the ultra-violet rays reaching the earth from the sun decreases rapidly when the sun departs from the zenith, and that this decrease is most marked with the short wave-lengths. Tisdall and Brown concluded, as the result of their experiments, that a marked increase occurs in the antirachitic effect of sunshine when the sun reaches an altitude of 35 degrees or more, that severe rickets is chiefly encountered in those cities where the altitude of the sun is below 35 degrees for some months of the year, and that therefore the period of the year during which rickets will probably develop can be calculated for any city in the world.

A study of the geographical incidence of rickets is also recorded by E. A. Smith (1927), with special reference to its occurrence in Utah. Sunlight is plentiful in this region, being 45 per cent. of the possible even in December to February, whilst the town stands at an altitude of 4310 feet above sea-level. The case incidence of rickets was 18.2 per cent. as compared with 50 to 80 per cent. amongst the poorer classes of the east coast cities. Smith suggests that the difference is accounted for by the better hygiene, the more abundant sunshine, and the increased ultra-violet light radiations at the higher altitudes where these observations were made.

Ultra-Violet Rays and Window Glass.—Ordinary window glass has been found by many observers to absorb completely the ultra-violet rays below about $310\text{ }\mu\mu$. The experiments of Fleming (1928) and of Fuchs and Priesel (1928) on rats, and of Bethke and Kennard (1927) on chicks, emphasise this deficiency of window glass. In the experiments of the latter workers, chicks, with unlimited access either to direct sunlight or to sunlight passing through a "screen glass" substitute, made equally good gains in weight, and did not develop leg weakness. Others under identical conditions, except that they received light through window glass, developed leg

weakness. Exposing chicks with leg weakness to direct sunlight, sunlight transmitted by a "screen glass" and a "fabric glass" substitute for 15 minutes, and 5 minutes to rays from a quartz mercury-vapour lamp, daily for 4 weeks, caused a marked improvement in behaviour, with a disappearance of leg weakness and increased ash content of the tibias.

Tests carried out by Brown and Tisdall (1927), with various special glasses transparent to ultra-violet rays, convinced them that for glazing ordinary windows they were of little value, owing to the small amount of sky-shine that is available. To be effective, these glasses should be used in the construction of special solaria, where both direct and diffuse light can be made use of. Sunlight filtered through corning glass, vioray, and vitaglass was shown to have from 25 per cent. to 50 per cent. of the antirachitic value of unfiltered sunlight.

The United States Bureau of standards finds that the transmission of ultra-violet rays by special glasses decreases with time. A sample of vitaglass, which had been in a hospital window in Rhode Island for a year, was found to have a transmission of 25 per cent. at $302\text{ }\mu\mu$ (for thickness of 2.3 mm.). Helioglass was found to decrease in transmission at about the same rate. Cel-o-glass (cellulose acetate), which when new transmitted 30 per cent., after being exposed to daylight for 8 months, transmitted only 5 to 10 per cent. Corex glass and quartz-lite appear to undergo no appreciable deterioration when exposed to solar radiation, and quartz-lite is affected only slightly by the quartz-mercury arc, but the transmission of corex is markedly decreased by the quartz-mercury arc. The thinner the glass, the greater in general is its transparency to ultra-violet rays.

Fleming (1928) has pointed out that a thin film of dirt on vitaglass cuts off 13 per cent. of the rays which it transmits when clean.

Transmission of Ultra-Violet Rays by Clothing.—Hess and Weinstock (1923) have carried out tests to ascertain whether it is necessary for the rays to impinge directly on the skin, employing as filters cotton and woollen goods of various mesh, using the carbon arc lamp as the source of radiation. It was found that if this cotton fabric was interposed, animals could be protected by three units of radiation, whereas if the material was somewhat heavier only slight protection could be accomplished with this intensity. White material permitted the passage of rays to a degree greater than black material manufactured by the same looms. Cotton and woollen stocking material, such as is commonly worn by infants, permitted but a small degree of the ultra-violet light to permeate unless very large dosage was employed. These experiments may be summarised by the statement that radiations must not necessarily impinge directly on the surface of the skin, but that there is—as might be expected—a quantitative relationship between the texture of ordinary wearing apparel and the degree to which it is pervious to the effective light rays.

(8) **VITAMIN D AND PHOTO-ACTIVITY.**—Many substances, including cod-liver oil, vegetable oils, and, according to Takahashi, Hamano, and Beck (1926), cholesterol, have been shown to possess after irradiation the power of blackening photographic plates placed near them. However, antirachitic action is not an effect of nascent H, but is due, it is conjectured, to the giving off of active O—a property possessed by all antirachitic and photo-active substances. Photo-activity represents the chemical action of gaseous products of the radiated substances upon the photographic plate, and is possessed, or may be attained, by easily oxidisable compounds, which, because of their unsaturated character, can, under the action of ultra-violet, easily form oxides and peroxides. These, on breaking down, yield oxygen atoms and, in the presence of moisture, strongly photo-active H_2O_2 which is present in a number of photo-active substances, and which causes reduction by giving off nascent H.

Several observers, including West and Bishop (1925), Farrington and Fosbinder (1925), Yoder (1926), Vollmer (1927), Cluzet and Kofman (1929), have shown that photo-activity does not depend on any rays given off by the substance, but is caused by hydrogen peroxide

either originally present, or produced under the influence of ultra-violet rays. While all the substances (irradiated oils, foods, and cholesterol, etc.), which have been shown to cure rickets, possess more or less photo-activity, there are a great many chemical compounds which possess this photo-activity and cannot cure rickets. There is, therefore, no essential relationship between photo-activity and the antirachitic factor. Neither is the fluorescence exhibited by various specimens of irradiated ergosterol connected with the formation of vitamin D, since Rosenheim (1927) obtained preparations of ergosterol under certain conditions which did not fluoresce when exposed to ultra-violet light, but which were nevertheless rendered as highly antirachitic as the fluorescent specimens by this exposure.

(9) **VITAMIN D AND CATHODE RAYS.**—Knudson (1929) has shown that commercial cholesterol exposed to cathode rays for 30 seconds is rendered antirachitic, while cholesterol, purified by the dibromide method, could not be rendered antirachitic either by cathode rays or ultra-violet radiation. The potency conferred by cathode rays, however, was not so great as that obtained by ultra-violet irradiation. These experiments indicate that the antirachitic properties produced by cathode rays are not due to exposure to ultra-violet radiation produced by the rays themselves, the highest potency obtained by cathode ray exposure being 0.0005 mgrm. per day and by ultra-violet irradiation 0.00002 mgrm. per day.

(10) **VITAMIN D AND RADIUM.**—That ergosterol can be converted into vitamin D by radium emanation is indicated by the experiments of Maisin, Mund, and co-workers (1930). Rats fed on a basal rachitogenic diet to which was added 0.1 mgrm. of ergosterol, exposed to radium emanation, failed to develop rickets after 39 days, while controls fed on the same diet with 0.1 mgrm. of unirradiated ergosterol became rachitic.

In a curative experiment, ergosterol exposed to radium emanation healed rickets, though its action was slightly less potent than that of ergosterol exposed to ultra-violet light.

The method of radium irradiation consisted in dissolving 0.03 gram of ergosterol in 30 c.c. of paraffin and exposing it to the action of 65 millicuries of radium in a sealed glass ampoule.

XXXI. PHYSIOLOGICAL ASPECTS OF VITAMIN D DEFICIENCY—RICKETS.

The part played by vitamin D in the bodily economy, though not yet completely elucidated, is undoubtedly related to the incidence of rickets. The factors concerned in metabolism in this disease are those which influence the laying down of Ca phosphate in bone, and their connection with vitamin D lies, according to recent researches, in the varying degree of absorption of calcium and phosphorus from the intestines. This absorption is determined by the correct hydrogen-ion concentration of the lumen of the intestine, maintained, in some unknown way, by the presence of vitamin D. From recent experiments carried out by Brown and Shohl (1930) it becomes clearer that vitamin D controls the intermediary metabolism of calcium and phosphorus. The mineral content of the bones is the resultant of the two actions of dissolution and deposition of the bone salts, controlled by vitamin D. What is equally important is that calcium and phosphorus must be present in the diet in sufficient amounts and in appropriate relationship to each other before proper bone growth or calcification can occur. No amount of vitamin can correct an absolute lack of bone-building salts.

(A) **BONE LESIONS IN RICKETS.**—The term "rickets" was first applied to the clinical picture resulting from the changes in the gross form and architecture of the bones. With the development of X-ray and histological technique, a definite standard of change is now recognised, and the lesions of true rickets can be distinguished from those of similar conditions occurring in osteoporosis, renal and coeliac infantilism, etc.

(1) **Radiographic Appearances.**—The characteristic appearance of the rachitic metaphysis

in what Wimberger (1925) has called active or florid rickets is an increase of distance between the epiphysis and diaphysis, with an irregular frayed edge in place of the sharp epiphyseal line. The end of the diaphysis is expanded laterally and scooped out, giving the "champagne glass" appearance. In very severe cases, when the bones can no longer bear the weight of the body, the distance between the epiphysis and diaphysis is still greater, but the irregular fraying is not marked, and there is no lateral expansion or "scooping out."

When healing commences, a line of shadow, due to the formation of a zone of calcification, appears at a short distance from the end of the metaphysis, and, as improvement continues, the space between this line and the diaphysis is gradually filled in by bone in the form of striæ. The shadow of the whole bone increases in intensity, and the epiphyseal line becomes more dense and regular until eventually the end of the bone shows a normal appearance. As the bone heals, the subperiosteal osteoid tissue also ossifies and shows as a shadow along the shafts of the long bones.

(2) **Histological Appearances.**—A longitudinal section of a long bone or costochondral junction of a healthy young animal shows a straight and sharply defined line of junction between the cartilage and the epiphysis (epiphyseal line); the zone of calcified cartilage, which is regular, forms a dense layer, with the axes of the trabeculæ parallel to each other and to the long axis of the bone. The rigid plate so formed keeps the growing cartilage in its place and regularises and controls its size, which would appear to be the primary determining factor in the growth of the bone.

In a rachitic bone the line of cartilage junction is irregular and convex towards the bone. The zone of calcified cartilage is absent and has merged with the frayed end of the diaphysis, which is more vascular than normal, and is scooped out and expanded laterally, the malformation showing itself clinically as an enlargement of the epiphysis. The microscopical appearances of normal bone emphasise the irregularity and disorder of the calcifying process in rickets. In normal bone the cartilage cells in the zone of cartilage proliferation are arranged in regular columns separated from each other by columns of hyaline matrix. Lime salts deposit themselves in the matrix columns, which therefore become rigid, and the cartilage cells are either destroyed by erosion or lost in the medullary spaces. Osteoblasts now arrange themselves round some of the calcified columns and form a narrow layer of osteoid tissue, which at first contains no calcium, but in which it is later deposited, with the formation of true bone. In rachitic bone this orderly arrangement no longer exists. Instead of the regular columns of cartilage cells in the zone of cartilage proliferation consists of an irregular assemblage of masses of cartilage partly calcified, partly uncalcified, and of bone trabeculæ forming an irregular network of osteoid tissue, the layer of which is greatly thickened, up to tenfold, according to Skaar and Haupl (1929). In its deepest parts, bone is irregularly deposited, giving rise to the frayed appearance of the diaphysis. The spaces between the masses of cartilage cells and the bone trabeculæ are filled, not with true cellular bone marrow, as in normal bone, but with a variety of fibrous tissue. It would seem that the characteristic disorder results primarily from the lack of calcification of the zone of hyaline cartilage. If this is soft, the growth pressure of the proliferating cartilage causes it to bulge, further weakening it until the cartilage bursts through. Then the orderly purposive growth of the bone is interfered with, and tissue growth from a zone of cartilage cells greater in size than normal takes place perpendicularly to the surface of the cartilage from a greater area. These secondary changes are interpreted by Skaar and Haupl (1929) as mechanical factors resulting from the softening of the skeleton. The fibrous marrow is similar to that in osteitis fibrosa. The localised marrow hæmorrhages are traumatic in origin and result in the development of richly cellular granulation tissue containing giant cells.

(3) **Rachitic Bone in Animals.**—(a) **IN THE RAT** the lesions produced by feeding with Pappenheimer's low phosphorus rickets-producing diet are not, according to Dobkevitch and Moulouquet (1929), identical with those met with in human rickets. Instead of the abundance of osteoid tissue, mixed up with a disorderly arrangement of cartilage and bone, characteristic of human rickets, the cartilage in rats undergoes great hyperplasia and is completely separated from the bone of the metaphysis by a zone of necrotic tissue (Schultz (1929)). By giving alternately low phosphorus diet and a normal diet, however, Dobkevitch and Moulouquet have succeeded in producing in the rat a histological picture closely resembling that of human rickets. Chick, Korenchevsky, and Roscoe (1926) have also found that rats fed on a low phosphorus diet (deficient also in vitamin D) develop rickets, with excess of osteoid tissue, increased area of proliferating cartilage, and defective deposition of calcium in the zone of provisional calcification.

(b) **IN THE CHICKEN.**—The lesions which occur in the legs of birds deprived of vitamin D are not associated with the typical bony changes and presence of excess of osteoid tissue. The alteration in the breadth of the histological zones is the only certain indication of disordered ossification, and the histological picture is difficult to identify with true rickets. If the chickens are allowed, however, to grow normally for 5 weeks, and are then put on a rachitic diet, the changes produced are more typical of true rickets. These changes include excess of osteoid in the spongiosa and absence of calcium in the zone of provisional calcification.

Nonidez (1928) found an excessive amount of fluid in the marrow of the rachitic area and marked distension of the lymphatics, together with other peculiar cellular changes. These changes suggest an alteration in the permeability of the capillary walls of the growing vessels which may affect the physico-chemical equilibrium that regulates the passage of calcium and phosphorus to the metaphyseal area. In contrast to rickets in other animals, the calcium content of the blood is low in chickens—a fact which may account for the great enlargement of the parathyroids which occurs when these birds are deprived of vitamin D. These experiments make it very probable that the leg weakness of chickens has the same significance as ordinary mammalian rickets.

(c) **IN THE PIG.**—Typical rachitic lesions have been demonstrated by Elliot and co-workers (1922), and by Zilva and co-workers (1921–1924), in young pigs subjected to dietary deficiencies.

(d) **IN THE RABBIT.**—Studies in rickets have been carried out, using the rabbit as an experimental animal, by Goldblatt and Moritz (1924), and by Kawamura and Kasama (1925). The lesions were characteristic of rickets and were associated with a disturbance of the calcium and phosphorus metabolism.

(e) **IN THE MONKEY.**—According to Christeller (1922), typical rickets and osteomalacia develop spontaneously in monkeys.

(4) **Fœtal Rickets.**—Whether rickets is actually present in the foetus has been questioned by some workers.

Kassowitz (1913) published sections of bones of newborn children, showing lesions which he considered those of true rickets, but Schmorl (1909) and Korenchevsky (1922) did not consider the results of their investigations conclusive, and Hess in his book *Rickets, including Osteomalacia and Tetany* (1930) also leaves the question open.

Preston Maxwell (1930) has recently presented studies of 2 cases which he states are undoubtedly fœtal rickets, occurring in children whose mothers were the subject of osteomalacia. The chief histological changes found in the ribs consisted of great deficiency in provisional calcification of cartilage at and for some time before death; irregularity of endochondral ossification from the diaphysis for a similar period before death, general osteoporosis, and absence of osteoid tissue in certain areas. On account of the last two changes, which are

characteristic of Barlow's disease (scurvy rickets), Turnbull, who examined the sections, considers that the condition of one of these cases was a mixture of rickets, or osteomalacia, with Barlow's disease.

(5) **Healing of Bone in Rickets**—(a) **RADIOGRAPHIC EVIDENCE**.—When healing commences, a somewhat ill-defined calcification shadow (a light strip on the film) appears near the proximal end of the metaphysis. This line is separated from the epiphysis by an uncalcified zone which appears black on the film, the extent of the zone depends on the width of the band of rachitic tissue, and the line of calcification does not always extend horizontally the whole width of the epiphysis, a gap sometimes being left in the middle. As improvement continues, the area of calcification extends, or two separate lines may appear, the space is gradually filled in, the epiphyseal line becomes more dense and regular, until eventually the whole of the metaphysis shows calcification and the picture is practically normal.

(b) **HISTOLOGICAL EVIDENCE**—THE "LINE TEST."—The "line test" of Shipley, McCollum and co-workers (1922), has provided a delicate reaction as evidence of healing under suitable conditions.

The following description of it is given by Bills, Honeywell, and McNair (1929), as used in their tests: "A robust and parasite-free breeding stock is maintained on either the stock diet of Steenbock (1923) (Diet 2965) or on a convenient modification of it. The young rats are weaned when 24 days old, when they weigh 35 to 40 grms. These young rats are placed in a room from which all daylight is excluded and kept for 18 days at 22–28° C. on McCollum's diet 3143, as follows:

Whole wheat flour	33
Yellow maize (finely ground)	33
Wheat gluten	15
Gelatin powder	15
Ca Carbonate	3
NaCl	1

"On the eighteenth day, or a few days afterwards, the substance to be tested is incorporated in Diet 3143 and administered for exactly 5 days, a daily food consumption record being kept. The rats must not eat less than 2 grms. in any one day, and not less than an average of 4 grms. per day, and they must not lose weight. According to Shipley's procedure, the proximal end of the tibia is sectioned longitudinally and exposed in 2 per cent. AgNO_3 to intense direct illumination under a low power binocular microscope. Upon the calcified areas Ag phosphate is formed and reduced to black silver. The principal criteria of healing are the development of the line at the zone of provisional calcification and the reappearance of bony trabeculae in the metaphyseal osteoid."

Under the high power the calcification is seen to take place in the matrix only, the cartilage cells being unaffected. The line of calcification does not disappear under the influence of sodium hyposulphite.

Van Leersum (1929) advises as control the preliminary microscopic examination of the epiphyseal line of the amputated leg of an animal before it is submitted to a rachitic diet. He also gives a warning against mistaking an abnormal deposition of calcium in the osteoid tissue at the epiphyseal junction for a normal zone of primary calcification.

Coward (1928) has shown the minimum amount of vitamin D required for a positive "line test" to be 2×10^{-8} grams.

A rickets-producing diet (Diet R and L) has been proposed by Randoin and Lecoq (1927),

who state that the usual rachitic diets are not completely satisfactory. Diet R and L consists of :

Meat peptone	17
Powdered brewers' yeast	3
Butter fat	5
Olive oil...	5
Saccharose	65
Pappenheimer's (Z 84) salt mixture	4
Ca lactate	1

This diet is stated to have a maximum deficiency in the antirachitic vitamin and a disproportion in the Ca : P ratio, but to contain all the other alimentary factors in normal amounts. This diet produces typical rickets in young white rats of 30 to 45 grams. in 18 to 20 days. Large rats (60 to 70 grms.) require 30 to 40 days. Animals losing weight during the test are to be discarded on account of the known healing effect of inanition. For protective or curative purposes the 5 (or even 2) parts of olive oil are replaced by cod-liver oil.

(c) **CHEMICAL EVIDENCE.**—Rachitic bones are characterised by a proportion of fat slightly greater than normal, a greatly diminished proportion of ash, and an increased proportion of "organic residue" (cartilage and connective tissue, estimated by subtracting the weight of the water, fat, and ash, from that of the fresh bone), and a greatly increased water content.

Chick, Korenchevsky, and Roscoe (1926) consider the best criterion of the chemical diagnosis of rickets to be the value of the ratio $\frac{\text{Mineral Ash}}{\text{Organic Residue}} \left(\frac{A}{R} \right)$.

In rachitic bone this ratio is about 0·4 to 0·8 as compared with osteoporotic bone 0·9 to 1·2.

(B) **METABOLISM IN RICKETS.**—The question of bone calcification is one which depends not only upon the presence or absence of vitamin D but upon the constitution of the body fluids with regard to calcium and phosphorus.

Most workers agree that the faulty utilisation of bone-forming elements in rickets is dependent on defective absorption, and that the prophylactic and curative effects of anti-rachitic agents are produced by the setting up of a condition which permits free absorption of calcium and phosphorus. Telfer (1926) has found, in all cases of rickets studied, a low retention of Ca, Mg, and phosphoric acid. The faecal output of mineral matter was higher than normal, and consisted chiefly of phosphate of lime. When healing occurred, retention of Ca and P increased and excess of mineral matter disappeared from the faeces.

The urinary phosphorus, which in rickets was less than the faecal, increased until it was higher than the faecal, and Telfer considers the change in ratio a valuable sign of healing.

The early workers on the subject had paid much more attention to the part played by calcium than to that of phosphorus. The simultaneous and independent investigations of two groups of American workers—McCullum, Simmonds, Shipley, and Park (1921–1922), and Sherman and Pappenheimer (1921)—gave due consideration to the importance of phosphorus, and Sherman and Pappenheimer more particularly proved that the form in which phosphorus existed—organic or inorganic—had a different effect in preventing rickets.

The question of the primary defect in rickets, whether impaired absorption phosphorus or calcium—is not yet entirely solved. Their metabolism is very intimately connected, and their balance in rickets variable, so that it is not possible to dogmatise on the theory of the determination of the laying down of calcium phosphate.

(1) **THE BLOOD IN RICKETS.**—The calcium content of the blood is very variable ; in severe rickets there is sometimes a negative balance, on the other hand, in quite severe cases

the balance is positive. Similarly, although rickets is generally associated with a low content of inorganic phosphate in the blood, the association is not frequent enough to be regarded as specific.

In Webster's and Hill's experiments (1925), rachitic rats, whose Ca and P was recorded for a month, had neither a negative calcium nor phosphorus balance; and Telfer (1926) confirmed this observation in rachitic infants.

(a) *Views in favour of Error in Phosphorus Metabolism.*—Shipley, Kramer, and Howland (1926) have stated that in uncomplicated rickets there is regularly a low concentration of inorganic phosphorus in the serum, 1.5 to 3.5 mgrms. per 100 c.c., as opposed to 5 to 6 mgrms. normal. They have also shown that rats can be made rachitic by feeding on a low phosphorus (McCollum's 3143) diet. The latter produces severe rickets regularly, and the serum is low in phosphorus (2.5 to 3 mgrms. per 100 c.c.). Shohl and co-workers (1927) have also postulated a close connection between rickets and a low phosphorus retention. During an experiment by Karelitz and Shohl (1927) in which rats were fed on a diet in which the ratio of Ca : P was 4.25 (the optimum ration for Ca deposition is 0.95), the blood serum showed a large decrease in phosphorus content, with a normal or slightly elevated calcium content. In a later experiment by Shohl and Bennett (1928) it was shown that even on a diet high in P and low Ca (Ca : P ratio, 0.66) the most marked deficiency lay in the P retention. They found also that the addition of phosphate to the diet of rats made rachitic by a high calcium, low phosphorus intake was followed by a rapid deposition of lime in the bones, and by an extremely high inorganic phosphorus and low calcium in the serum.

C. T. Williams (1928), in addition to confirming other workers' statements that the lowest blood phosphorus is found in children with active rickets, found that the blood phosphorus in children was higher at all seasons of the year in New Orleans, where severe rickets is rare, than in New York, with a considerable difference between the winter levels in the two regions. He also confirmed the presence of a normal seasonal variation in the blood phosphorus, the ebb of the phosphorus tide beginning in November, reaching its lowest level in February, and increasing then to reach the constant summer level in May.

Warkany (1930) believes that the function of vitamin D is to increase both the absorption of phosphorus and the retention of phosphorus in the blood. In studying the blood phosphorus of rachitic children he found that after the administration of the curative dose of irradiated ergosterol, the low phosphorus values rose to normal, and in 10 to 14 days were above normal, this high value often preceding any signs of healing as determined by X-ray photographs.

(b) *Views in favour of Error in Calcium Metabolism.*—Other workers, on the other hand, suggest that the abnormal excretion of P in rickets may be secondary to an error in Ca metabolism, and not necessarily to an impaired power of absorbing phosphorus as such.

Murdoch (1927) found that a diet poor in calcium and rich in phosphorus (added as NaH_2PO_4) gave rise to an increase, though not beyond normal limits, in the phosphorus content of the serum. Addition of calcium in excess to the diet they induced a fall in the serum phosphorus, a fall which was more marked in the rachitic than the normal children. The phosphorus absorptive power, as indicated by the rise in the serum phosphorus at hourly periods after the ingestion of 4 grms. of NaH_2PO_4 , was investigated in 11 children—5 normal, 4 with active rickets, and 2 showing healing rickets. In active rickets the absorption was similar to that of the normal cases, while in healing rickets it was abnormally high. From these facts Murdoch inclines to the conclusion that defective calcium absorption, and not disturbed phosphorus metabolism, is not at fault in rickets.

The observations of Courtney and co-workers (1928) lead them to believe that the calcium absorption or excretion in relation to rickets may not be so intimately associated with the phosphorus as is generally considered. They found that the total calcium content of the

cæcum of rats fed on McCollum's rachitogenic diet and kept indoors was much higher than that of rats fed on the same diet and exposed to sunshine. The calcium bound with phosphorus was essentially the same in the cæcum of the two groups of rats fed on the rachitogenic diet. They suggest, therefore, that the difference in the calcium concentration in the cæcum in these two groups is due to calcium not bound with phosphorus. No essential difference was found in the phosphorus concentration in the large intestines of the two groups of rats fed on the rachitogenic diet.

Daniels and co-workers (1929) have also come to the conclusion that the imbalance in the amount of calcium and phosphorus retention in rickets is due more frequently to a deficiency of calcium. From their experiments on infants they state that a calcium-phosphorus retention ratio of approximately 2 (calcium-phosphorus = 2) would seem to be normal for infants under 1 year of age. For optimum growth, infants between the ages of 2 and 9 months should retain between 40 and 50 mgrms. of calcium and between 20 and 25 mgrms. of phosphorus per kilogram of body weight. A high percentage of phosphorus in the stools of infants receiving feedings of cow's milk has been shown to be correlated with a low absorption of calcium. By determining the percentage of the ingested phosphorus in the urine and fæces, it is believed that early metabolic disturbances incident to the development of rickets in the infant receiving modifications of cow's milk may be detected.

Koch and Cahan (1927) also conclude from their experiments that the inorganic blood phosphate cannot be used as an infallible criterion for determining the antirachitic properties of a diet, since it was possible to obtain a low phosphorus value and a low product of calcium \times phosphorus in animals showing perfect calcification of the bones.

Skaar (1927), while supporting their conclusions, considers that there are two rachitic processes, the one where calcium metabolism is affected primarily, the other where the phosphorus metabolism is first affected. Cod-liver oil given with the diet causes the blood calcium and phosphorus to return to the normal, and the balance of these elements becomes positive. If 2 grms. of Na_2HPO_4 (0.17 gram P) is added to the rachitic diet daily the clinical signs of rickets are greatly aggravated, whilst the calcium and phosphorus balances rapidly decrease (serum phosphorus is lowered 50 per cent.).

From their more recent investigations (1929), Skaar and Haupl conclude that in some individuals the disturbance concerns chiefly the Ca metabolism, in others the P metabolism.

A conclusion which was fairly widely accepted until a doubt was cast upon it very recently by the investigations of Hess and co-workers (1930) (see below) is that of Howland and Kramer (1926), who believe the most valuable criterion of rickets to be the calcium phosphorus product.

According to their theory, any value of the product of the concentration of total phosphorus and total calcium in the serum, expressed in milligrams per cent., in children below 40, and in rats below 60, constitutes rickets.

A curious phenomenon has been observed by Hess, Lewis, and Rivkin (1929) in connection with the inorganic phosphate of the blood during dosage with small amounts of ergosterol, which points to the necessity of modifying this conclusion in certain cases. These workers found that "when inadequate amounts of antirachitic agents, for example, of irradiated ergosterol or of irradiated dried milk, were given as preventive measures, and when rickets developed—as evinced by the X-ray and clinical examinations—concentrations of 6 grms. or more of inorganic phosphorus and of 10 mgrms. or more of calcium per hundred cubic centimetres—in other words, normal values—were found in the blood." It was furthermore emphasised that this reaction is an early phenomenon that is "apt to occur a few weeks after the drug has been given."

It is evident from data given in the most recent experiments of Hess and co-workers (1930)

that it is misleading to rely on the calcium phosphorus product of the blood as an absolute criterion of rickets.

In all their cases, treated with viosterol, there was a product of more than 40, which is supposed to be indicative of an absence of rickets. The most marked instance, showing x-ray evidence of slight to moderate rickets, presented a calcium concentration of 10.8 grms. and an inorganic phosphorus of 6.5; in other words, a product of more than 70.

Hess suggests that the most probable explanation of this association of rickets with a normal serum phosphorus is that in the course of irradiation a factor is produced which stimulates phosphatæmia, quite apart from any rachitic action.

György's theory (1929) is an interesting one, but his view of the pathogenesis of rickets is not universally accepted. He emphasises the fact that the disturbances in the blood calcium and phosphorus are merely indications of the aberrations in the intermediary metabolism of the cells and tissues. He believes that there is a condition of acidosis arising in the tissues of the rachitic individual, due probably to a slowing of the process of metabolism resulting in the accumulation of acid metabolites. This slowing of the metabolic process is dependent on a diminished oxidation in the tissues.

Freudenberg and György point out that the phosphorus ion is concerned with the acceleration of intracellular oxidative processes; but György indicates that the exact mechanism and relation of intermediary metabolism to the disturbed blood calcium and phosphorus and to the process of ossification is as yet an unanswered question.

(2) THE CEREBRO-SPINAL FLUID IN RICKETS.—According to Macchi (1929), the variations in the Ca and P content of the cerebro-spinal fluid in children follow more or less closely those of the blood, but to a less degree. In the case of calcium the average content fell during treatment, the maximum being 7.3, and the minimum 5.5 mgrms. at the commencement, and 6.5 and 5.4 mgrms. per cent. respectively at the termination. In the case of phosphorus the average content rose; the initial maximum and minimum were respectively 1.50 and 1.22, and after treatment 2.50 and 1.94 per cent.

De Toni (1930) states that the cerebro-spinal fluid of infants with rickets has an average content of lactic acid and of inorganic phosphorus much lower than that of normal infants.

(3) CARBOHYDRATE METABOLISM IN RICKETS.—It is stated by Baldwin, Nelson, and McDonald (1928) that the antirachitic factor is requisite to normal carbohydrate metabolism in the chick. They found that prior to the appearance of the characteristic symptoms of rickets (after 15 to 18 days upon a vitamin D-deficient diet), a pronounced fall in the level of the respiratory quotient took place. This would seem to indicate a rapid loss in ability to utilise carbohydrates as a chief source of fuel. During the time that symptoms of leg weakness were present, the respiratory quotient was indicative of the utilisation of fats alone, although carbohydrates composed 47 per cent. of the diet. Restoration of the antirachitic substance, either by the inclusion of cod-liver oil in the diet or by irradiation, brought the respiratory quotient to unity.

A disturbance of the carbohydrate metabolism in rickets has been observed by Freudenberg and György (1920), Freudenberg and Welcker (1926), Adam (1927), and König and Lenart (1927), signalled by an increase in the amount of diastase in the urine and fæces, and a decrease of the glycolytic power and the lactic acid level of the blood serum. König and Lenart found an apparent defect in the blood sugar regulation, a steeper alimentary glycaemia curve in children with rickets than in controls. The hyperglycæmia also lasted longer than in the normal children.

De Toni (1930) has also found the experimental hyperglycæmia somewhat increased and of longer duration in infants having rickets than in normal children, but does not consider the test of alimentary hyperglycæmia reliable.

The "acidosis" theory of rickets has been brought forward by Freudenberg and György and supported by other workers. The assumption is that though the hydrogen-ion concentration of the blood has been shown to be normal in rickets, a compensated acidosis is present, in that there is a lowering of the alkaline reserve of the organism.

Eric Pritchard (1919) has suggested that in rickets, calcium (available base) is withheld from developing bone, since it becomes more urgently required to neutralise acid products of incomplete oxidation. His suggestion is substantiated by the fact that there is in rickets an increased urinary excretion of phosphates, and an excretion of creatine, as shown by Schwarz (1910) and by Underhill (1916), who found it in conditions of acidosis when carbohydrate metabolism was unaffected. Dr. Amy Hodgson (1921) attempted to test the lowering of the alkaline reserve in rickets by comparing the proportion of nitrogen excreted as ammonia relative to total nitrogen excretion in the urine of normal and rachitic children. Her experiments were somewhat incomplete, but suggest that there is some indication of a lowering of the alkaline reserve in active rickets.

The presence of an acidosis in rickets in dogs has also been reported by Liégeois and Lefèvre (1929). In their experiments the mean blood pH was 7.36 (normal, 7.40); the mean alkaline reserve (total CO_2) was 46 (normal 54); the mean $H_2CO_3/NaHCO_3$ ratio was 1/18.4 (normal 1/20.3). Analogous findings were obtained in a dog with osteomalacia. There was no intestinal trouble in any of the dogs which might lead to direct loss of basic radicles through diarrhoea or by fixation of excess acid in the intestine. When the rachitic dogs were treated with irradiated ergosterol (vigantol) the acidosis commenced to diminish in a week's time, and gradually returned to normal. The blood pH reached a figure slightly above normal, whilst the alkaline reserve tended to remain at a figure slightly below normal. The diminution of the acidosis was maintained after treatment was suspended. Similar results were observed on treating the dog suffering from osteomalacia.

Ullrich (1929), supporting the acidosis theory, correlating the acidosis with a hypophosphatæmia, which he states is caused as follows: "Since the ratio $Ca : P$ is higher in the zone of growth than in mature bone, in the growing organism the rachitic acidosis causes an endogenous hypercalcæmia, and the elimination of this excess as acid phosphate to compensate the acidosis, results in hypophosphatæmia."

(4) **FAT METABOLISM IN RICKETS.**—There is practically no disturbance of fat metabolism. According to Hutchison (1920), fat splitting is adequate and normal, but Telfer (1926) reports that although concentration of fat in the fæces tends to be normal, combined fatty acids are relatively low.

(5) **CHOLESTEROL METABOLISM IN RICKETS.**—A hypocholesterolæmia has been found to be a constant feature in rickets. Dorlencourt and Seisoff (1929) have found the proportion of cholesterol in the blood in rickets to be 0.83 per cent. in place of 1.40 per cent., the normal figure, and this proportion rises as the rachitic condition improves.

Similar figures are reported by Lesné, Sylvestre, and Zizine (1929), but they found varying results with cod-liver oil treatment. Sometimes the blood cholesterol was increased, sometimes decreased, by administration of cod-liver oil.

(C) **MECHANISM OF THE DEPOSITION OF CALCIUM PHOSPHATE.**—Several theories have been advanced to account for the non-calcification of bone in rickets, or rather to answer the question as put by Freudenberg: "How does the phosphatæmia develop under the influence of a deficiency in light and vitamins?"

Freudenberg himself (Freudenberg and György (1923)) believes that the protein of cartilage specifically adsorbs calcium salts, forming a calcium-cartilage complex. When phosphorus is added, the combination becomes a calcium-phosphate-cartilage complex. His theory is supported by the fact that cartilage ossifies when placed in solutions of calcium

phosphate. It does not, however, explain satisfactorily why calcification does not occur in other cartilage besides that of bone.

The theory of Shipley, Kramer, and Howland (1926) inclines to regard the calcification process more from the aspect of salt balance than vitamin action.

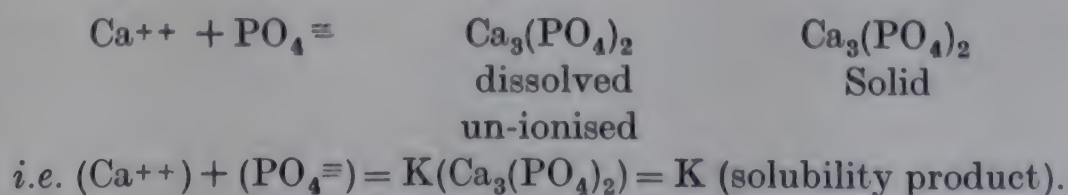
Their studies on calcification *in vitro* show that the defect in rickets does not lie in the bone itself, and that rachitic cartilage can calcify if in contact with normal serum.

Shipley observed in 1924 that when cartilage and bone of a rachitic rat are incubated in the serum of rachitic rats, no calcification occurs. On the other hand, pieces of cartilage and bone from the same animal incubated in the serum of normal rats show calcification similar to that in an animal undergoing cure.

Later studies were undertaken to determine the calcification in solutions of inorganic salts of Ca and P. The calcium in these solutions (which contained about 0.03 per cent. sodium carbonate, N/10 NaCl, and 0.0016 MgSO₄) varied from 5 to 10 mgrms. per 100 c.c., and the inorganic P from 1.5 to 9 mgrms. per 100 c.c.

It was found that, with a Ca concentration of 10 mgrms., calcification occurred in 24 hours with a simultaneous P concentration of 3.5 mgrms., and in 9 hours with a P concentration of 4 mgrms. If the Ca concentration was 5 mgrms., calcification occurred when the P process was 7. Shear and Kramer (1928) have found that calcification is not obtained when the empirical calcium phosphorus product is less than 30. Calcification was delayed by the addition of egg-albumen, and completely inhibited by heat, by cold, and by protoplasmic poisons. Shipley and co-workers suggest that these experiments show the selective deposition of Ca salts to depend upon the activity of living tissue, and that the failure of calcification in rickets does not depend upon any primary inability on the part of rachitic bone or cartilage, but upon an insufficient concentration of the necessary elements in the fluids bathing these tissues.

Robison (1924, 1926) has advanced a hypothesis that calcification depends upon the activity of an enzyme secreted by the osteoblasts and hypertrophic cartilage cells. He had in early experiments (1924) shown that the bones and ossifying cartilage of young animals contained an enzyme which rapidly hydrolysed hexose-monophosphoric ester and glycerophosphoric ester with liberation of free phosphate ions. This hexose-monophosphoric ester was isolated by Robison from the products of the fermentation of sugar by yeast, and its calcium salts are very soluble. The kidney and intestinal wall were also found to have the power of breaking down hexose-monophosphate, the optimum reaction taking place at pH 8.4. On both sides of this pH the activity diminishes, therefore the liberation of inorganic phosphate by the action of an esterase will not necessarily be followed by deposition of Ca phosphate in any tissue: the reaction is not under optimum conditions unless a high pH is present. For this reason Robison does not consider his theory in any way opposed to that of Shipley, Kramer, and Howland; he puts forward the suggestion that the osteoblasts and hypertrophic cartilage may have the power to raise the pH of the body fluids in which they are bathed. Even a small upward change would cause a precipitation of Ca₃(PO₄)₂ and Ca CO₃ in a solution saturated with respect to these salts. He considers the reactions taking place to be of the following character:



If the solubility product is greater than K (*i.e.* the product of the concentration of Ca and PO₄ ions sufficiently high), deposition of solid calcium phosphate will occur; if it is less than K, some of the solid will be redissolved. It will be observed that the equilibrium depends

upon the concentration of the ions of Ca and P, not directly upon the total Ca and P present. Both deposition and resolution occur in the body, the former during normal bone formation, the latter under special conditions, *e.g.* if the demand of the body for calcium is not satisfied by the diet.

The occurrence in bone and ossifying cartilage of an enzyme which hydrolyses hexose-monophosphate is not complete evidence of the share of this ester in the process of ossification. It is necessary to show its presence in the blood. This question Kay and Robison (1924) have investigated. They have found the hexose-monophosphoric ester present almost entirely in the corpuscles, but no conclusive evidence of its presence in the plasma. By the action of bone extracts on unclotted whole blood, the amount of inorganic phosphate in the plasma was increased. This probably indicates that the hexose-monophosphate is able to diffuse from the corpuscles to the plasma and that the former may act as reservoirs.

(D) PART PLAYED IN OSSIFICATION BY VITAMIN D.—The action of vitamin D as the essential factor in rickets appears to be closely connected with the hydrogen-ion concentration of the lumen of the intestine, the preservation by vitamin D of the correct balance of the *pH* determining the absorption of calcium and phosphorus from the intestinal contents.

Zucker (1923) first showed that the intestinal contents of animals on a rachitic diet had an increased alkalinity, becoming more acid when vitamin D was added to the diet. Bacharach and Jephcott (1926) confirmed these results, finding that the change towards acidity synchronised with the cure of the rachitic condition, and suggesting, therefore, a criterion for determining the clinical condition of the animal with regard to rickets.

Grayzel and Miller (1928) also found the alkaline effect of a rachitic diet in adult dogs to be very constant, the addition of cod-liver oil bringing back the intestinal reaction to the normal range. In their experiments the *pH* values were obtained both electrometrically and colorimetrically, the colorimetric result being in every case lower than the electrometric, the average difference being 0.2 of a *pH* unit.

In children, however, the correlation between the different stages of rickets and the *pH* value of the faeces has not been found so definite. Redman (1928) found great variations in the *pH* values of the faeces, determined by means of the quinhydrone electrode, in both normal and rachitic children with various treatments and at different stages of improvement.

The results of experiments made by Bauer and Marble (1929) show that there is a marked increase in calcium absorption from the intestinal tract when viosterol is administered. They consider that infantile tetany, rickets, and osteomalacia are all disorders of calcium metabolism in which the mechanism at fault is absorption from the gastro-intestinal tract.

The absorption of calcium and phosphorus varies in different parts of the intestine. Telfer (1926) has produced evidence showing that the absorption of the calcium only occurs in the upper part of the intestinal tract where the acidity due to the presence of gastric juice favours the solution of calcium salts in the food. When the intestinal secretions render the contents alkaline, calcium phosphate is precipitated in insoluble form.

Yoder (1927), who has confirmed these observations, suggests that the rise in *pH* of the alimentary contents, which occurs with a rachitic diet, may thus be an important factor in the deficient calcium absorption in rickets.

Bergheim (1926, 1928) also maintains that acidity, especially lactic acid fermentation, increases the absorption of calcium, and has shown that the administration of lactose promotes the absorption of calcium, whereas maltose and glucose scarcely affect it. His investigations show that the high-calcium, low-phosphorus type of rickets is not due to failure of Ca absorption, since rachitic rats, whether cod-liver oil is added to their diet or not, show considerable Ca absorption in the upper small intestine. That phosphorus is actually excreted in large amount into the small intestine under the influence of antirachitic agents has been shown

both by Bergheim and Yoder, and under these conditions there is an increased acidity due to the phosphoric acid. Yoder has stated that when cod-liver oil is the antirachitic agent, the increased acidity affects both the duodenum and the rest of the small intestine, whereas with irradiation the acidity occurs only below the duodenum, suggesting a distinction between the action of the two antirachitic agents. The composition of the primary calcification, however, appears to be independent of the antirachitic agent. According to Shear and Kramer (1928), high Ca:P ratios were obtained regardless of whether the fresh calcification was induced by cod-liver oil concentrate, butter, irradiated cholesterol, or irradiated yeast. When rachitic rats, in which this process of excretion of phosphorus into the small intestine is going on, are given cod-liver oil, they make up for the loss of phosphorus by increased absorption from the cæcum and large intestine. Rachitic animals which receive no curative agent have not this compensatory power, and therefore the phosphorus balance remains negative. Although calcium is absorbed in the upper small intestine in the low-phosphorus, high-calcium type of rickets, it is re-excreted into the lower bowel, and a subnormal calcium balance then occurs. This excretion, according to Bergheim, must be due to a failure of the bones to fix calcium, and he believes this failure to arise from an inability of the tissues to break down organic phosphate. He considers that the antirachitic vitamin in some way facilitates this action.

The suggestion that it is not the rachitic animal's power of forming bone that is at fault is supported by the experiments of McCollum and his co-workers on the effect of starvation in rickets; they took rats, which they rendered rachitic, and starved them for a few days and then killed them. Histological examination showed that the period of starvation was followed by a marked deposition of lime salts. The breakdown of the starving animal's tissues liberated phosphorus and calcium, which were promptly used to build up bone.

Bond (1929) has advanced a hypothesis which seems to support and extend Bergheim's view that lack of calcification in rickets is due to the failure of the bones to fix calcium. He believes that normally the bone cells are able to extract the calcium phosphate from the blood by virtue of the permeability of their pericellular and perinuclear membranes. He suggests that vitamin D acts on the lipoid rather than the protein elements in the cell, and in this way renders the cytoplasmic and perinuclear lipoid membranes more permeable. The increased permeability is accompanied by increased nuclear cytoplasmic interchange, and the result is the speeding-up of the calcium-phosphorus metabolism of the bone cells. The experiments by which he supports his conclusions are based on the effect upon lecithin of irradiated ergosterol. Lecithin, under the influence of hydration, shows marked activity in the formation of droplets and myelin threads. Films deposited from solutions of ergosterol and lecithin in chloroform showed much greater activity when the ergosterol had been irradiated. That the effect on the lecithin was due to the vitamin D and not to any peroxide of ergosterol, which formed on the irradiation of the sterol in contact with air, was shown by the following experiment: "When two films or smears of ergosterol were made on a glass slide and irradiated, one in the air and one through a film of water covered by a quartz slip, the air being thus excluded, only the irradiated sterol from the film protected against contact with the air activated the lecithin. The sterol irradiated in contact with air failed to do so."

In associating these findings with the changes which can be observed in living cells as the result of exposure to the action of irradiated sterol, Bond refers to his earlier experiments (1927), showing that living leucocytes, when incubated in blood or in blood serum on a thin film of irradiated ergosterol in a closed cell, respond sooner and more strongly than corresponding cells incubated on an ergosterol film which has not been previously irradiated. "The cells in the irradiated film show increased pseudopodial activity, and they elaborate droplets of iodophil substance sooner and in larger quantity than the cells in the non-irradiated film. This formation of iodophil substance depends on nuclear and cytoplasmic changes, especially

in the perinuclear lipid-protein membrane. The significance of this observation lies in the observed influence of certain fat-soluble substances on this perinuclear membrane, which is now believed to be composed, in part at any rate, of lecithin, possibly in combination with cholesterol and other proteid substances."

Parsons (1928), while agreeing with the greater part of Bergheim's theory, holds the view that the antirachitic vitamin carries out its action by maintaining in some unknown way the correct pH of the lumen of the intestine.

It must be stated, however, that while the researches of Redman, Willimott, and Wokes (1927) on faecal alkalinity have shown results which correspond with Bergheim's curve of calcium absorption, an investigation of Shohl and Bing (1928) appears to contradict their conclusions. Shohl and Bing have shown that the change from pH 7.4 to that pH 6.2 occurring with the administration of cod-liver oil to rachitic rats, as indicated by Zucker and Matzner (1923), does not occur when the rats are cured by irradiation of their food, or by the addition of alkaline phosphates. From this finding it would appear that the curve of rickets is not necessarily associated with an alteration of the pH of the faeces from alkali to acid. Finally, Drummond (1928) suggests that the essential action of vitamin D is to produce an increased permeability of the intestinal wall, which, with a corresponding alteration of the pH of the lumen, allows of the correct absorption of calcium and phosphorus for normal calcification of bone.

(E) VITAMIN D AND THE PARATHYROIDS.—Since both vitamin D and the parathyroids are known to influence calcium metabolism, numerous investigations have been undertaken with a view to tracing the connection between the two agents. Greenwald and Gross (1925) have advanced the view that vitamin D stimulates the parathyroids and that the parathyroid hormone in its turn increases the calcium-dissolving power of the plasma. Administering parathyroid extract increases serum calcium and mobilises more calcium, which in cases of prolonged administration must be withdrawn from the bones to equalise the correspondingly greater excretion of calcium.

In view of this relationship the difference in reaction to parathyroid stimulation in young animals and adults can be explained. The young animal has a constant need for calcium to form growing bone, and therefore can fix calcium as fast as it appears in the serum, so that an increase in the blood calcium means increased retention. The same process takes place in the lactating animal, in which also large amounts of calcium are removed from the blood. The adult animal, on the other hand, has only a smaller, steadier need for calcium, and therefore a temporary increase in the blood calcium means decreased retention and actual loss. This argument is supported by Greenwald and Gross's experiment (1928) on an adult dog, in which cod-liver oil had the effect of increasing calcium excretion, and by the experiments of Hess, Weinstock, and Rivkin (1929) on monkeys. In Hess's case the blood calcium was lowered by a calcium-deficient diet, and then irradiated ergosterol was administered. The blood calcium promptly rose to normal. When the parathyroids were removed, the blood calcium fell once more and could not be raised by any amount of irradiated ergosterol.

The reverse experiment has been performed by Morgan and Garrison (1930). Using young dogs, these investigators examined the power of parathyroid extract to raise the blood calcium level as affected by simultaneous presence of vitamin D in the body of the experimental animal. With diets otherwise adequate, a deficiency in the antirachitic factor prevented the hypercalcaemia usually observed following the injection of parathyroid extract. When both viosterol and parathyroid extract were given, extremely high concentrations of serum calcium were observed.

Higgins and Sheard (1925) have shown that chicks grown in light from which the so-called vital rays have been removed invariably exhibit a hyperplasia of the parathyroids. This

overgrowth can be prevented in these animals by subjecting them to direct irradiation containing the ultra-violet portion of the spectrum or by administering cod-liver oil, while Blunt and Cowan (1929) state that some cases of osteomalacia have been traced to a parathyroid tumour and cured by its removal. Greenwald (1928) has reported that the feeding of cod-liver oil does not prevent tetany in thyro-parathyroidectomised dogs.

(F) **OTHER VIEWS ON THE ÆTIOLOGY OF RICKETS.**—Whether rickets is entirely a deficiency disease, due to lack of vitamin D, with a consequent disturbance of the calcium and phosphorus in the blood, has been questioned by several workers, principally Mellanby (1926), and more recently Pfaundler (1930).

(1) **THE “RACHITOGENIC FACTOR” IN DIET.**—Mellanby believes that there is a toxic substance, occurring chiefly in cereals, the presence of which renders the foodstuff containing it actively rickets-producing, and that when the substance has been removed the same diet has no ill-effects.

Holst (1927) has brought evidence to support Mellanby's idea. He has stated that rats develop rickets when they are fed on an exclusive diet of oats or yellow peas, or wheat or rye, or barley or maize, or polished rice. Pure starch does not produce the effect; rats are able to live on it for some time, and develop only an osteoporosis. An addition of casein and lard makes the animals live longer and grow, and rickets is not produced, but when the casein and lard are added to the diet of pure oats or other pure foodstuffs, rickets is not prevented.

Holst found that he could extract the rachitogenic substance by boiling oatmeal with a dilute solution of hydrochloric acid; the extract, when filtered and neutralised and given to rats in a daily dose corresponding to 15 to 20 grms. of oatmeal, produced rickets in rats on the pure starch diet. The substance is organic since the ash of oats does not possess the rickets-producing power; it is dialysable and can be precipitated with alcohol, but Holst's results are not entirely in agreement with Mellanby's as to the physical and chemical behaviour of the rachitogenic substance.

Mellanby's more recent experiments (1930) have led him to conclude that cereals differ widely in their anticalcifying properties. Oatmeal, he states, is the worst, and white flour interferes least with the calcifying process. Intermediate between these are maize, rye, barley, and rice. The embryo or germ of wheat and maize, and probably of other grains, has this toxic effect more strongly developed than the endosperm. The germ of rye has vitamin D (in small quantities) as well as the substance interfering with calcification, and these actions can be separated owing to the solubility of vitamin D in alcohol. The substance in cereals having a toxic effect, though its chemical nature is still unknown, is not associated with the carbohydrate nor with the fat. Mellanby thinks it may be in some way bound up with a protein, but this is still to be proved. He emphasises the fact that the calcifying effect of vitamin D, whether it forms part of the diet or whether it is supplied to the body as the result of the activation by sunlight of ergosterol in the skin, will always, if present in sufficient quantity, antagonise the anticalcifying effect of cereals.

Further investigations into the nature of the anticalcifying substance in cereals, particularly oatmeal, have been made by Mirvish (1929). He has obtained an extract from oatmeal which he claims lowers the blood calcium after injection into rabbits. The extract was prepared by boiling oatmeal with 1 per cent. HCl for some hours, dialysing the concentrated solution against distilled water, evaporating the dialysate to dryness, extracting with 95 per cent. alcohol, when, after evaporating off the alcohol, the active residue is obtained. The response to the injection of this material is similar to that found by Mirvish after the injection of extracts of bovine ovaries, namely, a lowering of blood calcium of about 30 to 35 per cent. usually in 24 hours with a return to normal in about 48 hours. He concludes that the anticalcifying action of oatmeal is due simply to a reduction in blood calcium, and

suggests that this explains why the cereal effect can be antagonised either by giving calcium salts or vitamin D, both of which act by raising the blood calcium. He also considers that, in view of his findings, rickets cannot be conceived to be purely a vitamin deficiency condition. It may yet prove to be a manifestation of hypo-parathyroidism in young, growing animals.

Cowgill (1929) states that cereal germ has not only anticacifying properties but in large quantities a toxic action on the nervous system.

Hess and Weinstock (1927) have also suggested that a rachitogenic substance is present in cows' milk. They found that in New York, in March, all bottle-fed infants, unless they received some rickets-preventing factor, suffered from some degree of rickets. Only $\frac{1}{2}$ to $\frac{2}{3}$ of the breast-fed infants were found to suffer in this way. When they tested the antirachitic value of cows' milk and of human milk on rats, they found that 20 to 25 c.c. of the former were needed to prevent rickets, while 25 to 30 c.c. of human milk were needed. This result showed that the human milk tested was of very poor antirachitic potency for rats, whereas Hess and Weinstock make the assertion that breast milk is of high antirachitic potency for infants. They therefore conclude that "the high protective value of human milk in infantile rickets cannot be ascribed to its content of the antirachitic factor." They go so far as to state, from experiments on the cream and top-third of the milk, that there is a rachitogenic factor in the skim fraction of the top milk which offsets the antirachitic value of the cream contained in it, and presumably accounts for the inferiority of cows' to human milk in the prevention of rickets. Their conclusions have not, however, been supported by undeniable evidence.

Tisdall and Brown (1930) regard most cereals as rickets-producing foods, but consider that irradiation removes the tendency and renders them rickets-preventing. Their view is supported by the experiments of Steenbock, Black, and Thomas (1930), who found that in general the effect of irradiation was to equalise the calcification, but without calcium supplements the percentage of bone ash was not increased.

(2) CORRECTION OF AN ABNORMAL CONSTITUTIONAL FACTOR BY VITAMIN D.—Pfaundler (1930) does not agree that rickets is a pure avitaminosis, in the sense that it is caused by the absence of vitamin D from the diet. He points out that rickets does not occur among the infants of peoples, especially in the polar regions, who received neither antirachitic substance in their diet nor sufficient ultra-violet irradiation.

He admits, however, that the antirachitic substance indisputably prevents and counteracts rickets, whether administered by mouth or formed in the body by the action of ultra-violet rays. His theory is that in rickets an abnormal condition, caused by an ectogenic or endogenic factor, is constitutionally present, and this injurious factor is the primary cause of rickets. The action of vitamin D is apparently that of counteracting this condition rather than of primarily producing rickets by its absence.

XXXII. THE PREVENTION AND TREATMENT OF RICKETS.

Antirachitic agencies resolve themselves into three factors—(A) exposure of the rachitic subject to sunlight or ultra-violet irradiation; (B) administration of cod-liver oil; and (C) administration of irradiated substances, including irradiated ergosterol *par excellence*. The question of diet, in relation to its calcium and phosphorus content, is of secondary practical importance, since milk, which is the normal food of the young animal, usually contains a sufficient quantity of both elements.

Hess, Unger, and Pappenheimer (1921), and also Park, Shipley, McCollum, and Simmonds (1922) have shown that even if the diet contains less than 150 mgrms. of phosphorus per 100 grms. of diet, a quantity which invariably produces the low phosphorus type of rickets, exposure to sunlight is approximately equivalent to doubling the intake of phosphorus in

this dietary, and the same result can be brought about by using artificial sources of light.

(A) **EXPOSURE TO SUNLIGHT OR ULTRA-VIOLET IRRADIATION**—(1) **Sunlight**.—If infants are placed in sunlight for about $\frac{1}{2}$ hour daily so that the rays impinge directly on their skin, the rachitic lesions of the epiphyses will rapidly undergo calcification. In France this fact has been taken advantage of in establishing the prophylaxis of rickets.

Armand-Delille (1927) reports the results of sun treatment in the nursing clinic of the Salpêtrière where, over a period of 4 years, 194 children were given sun baths regularly during the months of April to October, the children being exposed naked whenever the weather was suitable. Debilitated children, born during the months of November and December, were also given baths of artificial ultra-violet light. Not one of these children developed rickets. Similar results have been obtained in other institutions in Paris. These clinics have been established in all large hospitals, and have proved very satisfactory both from a medical and industrial point of view, and form obviously a more humane way of dealing with working mothers' children than was formerly available. In Boston similar results were obtained by Wyman (1927) in children suffering from acute rickets, the test being carried out in the winter months, the infants remaining undressed all day and being placed directly against the windows in a warm room. The windows, however, which gave good results, were made of quartz and corning glass, which transmit the antirachitic rays between 290 and 310 $\mu\mu$.

SEASONAL VARIATION OF RICKETS.—A connection between the seasonal incidence of rickets and the antirachitic effect of sunshine has been traced by several observers. Rickets is known to occur most severely during the winter months of January, February, and March. It has been shown by several groups of workers that there is a coincident seasonal tide in the blood phosphate. Hess and Unger (1921) found that accompanying the manifestations of healing of rickets there was an almost constant rise of the lowered organic phosphate of the blood to the normal level, and that the seasonal tide (3.92 mgrms. per 100 c.c. of blood in December, decreasing steadily during January, February, and March, with a rise through April, May, and June to the level of 4.20 mgrms. per cent.) could be attributed mainly to the seasonal variation of sunlight.

This observation has been confirmed by those of Brown and Tisdall (1928) in Toronto. They found that there is a sharp rise in the antirachitic effect of sunshine about the beginning of March, and that the antirachitic effect of April and May sunshine is about 8 times that of December, January, and February. The same investigators also found that a considerable amount of ultra-violet irradiation takes place as a result of reflected rays from the sky and clouds, the antirachitic effect of which is approximately $\frac{1}{2}$ to $\frac{2}{3}$ of that produced by what is ordinarily termed sunshine—namely, direct rays from the sun plus reflected rays from the sky. In the table (p. 156) they have shown the changes produced in the inorganic blood phosphate and in the percentage of ash in the bones of rats fed on a rachitogenic diet and exposed to the sun's rays during the spring and autumn of 1927 and the spring of 1928.

Doubt has been cast by Havard and Hoyle (1928) upon the conception that the summer rise in the level of blood phosphate in adults is due to increased incidence of ultra-violet light. They state as the basis of their argument that neither the addition of irradiated ergosterol to the diet of a healthy adult for 21 days during the winter, nor daily irradiation of the subjects by carbon arc lamps, caused any significant change in the blood phosphate or calcium.

(2) **Ultra-Violet Light**.—In 1919, Huldschinsky, using the mercury-vapour lamp, noted definite healing of rickets by means of radiographs, and, in 1924, Hess was able to postulate a unit of protective irradiation, by exposing white rats, fed on a standard rickets-producing diet, to the mercury-vapour quartz lamp at a distance of 3 feet for a daily period of 3 minutes. Since then the use of ultra-violet irradiation in both the prophylaxis and the treatment of

ANTIRACHITIC EFFECT OF SUNSHINE ON RATS FED ON A RACHITOGENIC DIET.*

Rats Killed.				Rats Exposed to Sunshine.		Controls kept Inside.	
				Inorganic Phosphorus per 100 c.c. of Blood.	Percentage of Ash in Bone.	Inorganic Phosphorus per 100 c.c. of Blood.	Percentage of Ash in Bone.
1927.							
February	7	2.6	37.5	1.8	26.7
"	14	2.4	36.7	1.6	32.4
"	21	3.2	39.9	—	—
"	28	4.5	51.0	1.4	32.0
March	7	4.9	43.6	2.0	39.6
"	14	4.5	51.0	1.9	34.6
1928.							
February	6	2.1	38.0	1.6	35.6
"	13	2.6	35.7	1.3	29.8
"	20	1.9	37.6	—	27.0
"	27	2.6	43.4	1.3	27.1
March	5	5.4	48.9	—	32.7
1927.							
September	27	3.4	48.0	1.9	—
October	3	—	43.2	1.2	31.2
"	10	4.2	46.6	1.4	34.6
"	17	6.8	44.4	2.7	33.3
"	24	3.3	39.5	1.4	30.0
"	31	2.8	40.0	2.4	33.8
November	14	2.4	38.8	1.6	32.6

* The rats were exposed to sunshine two hours daily at midday for four weeks.

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rickets has been tested by many workers with not invariably, though generally, successful results.

In spite of the apparent discrepancy of these observations, the evidence in favour of the beneficial results of ultra-violet irradiation in rickets is too strong to be disregarded.

Selkirk and co-workers (1928), for instance, have given evidence, based on a study of 386 babies, that ultra-violet irradiation is a practicable method to use in the prevention of rickets. Clinical and X-ray examinations were made up to and including 8 months of age. When twins, premature and syphilitic infants, and babies lost from observation were eliminated, 237 cases remained for analysis. These were divided into four groups in order to eliminate the complicating factor of cod-liver oil. The infants in the group receiving irradiation and no cod-liver oil showed 17 per cent. of rickets, as compared with 56 per cent. of the control group who received no irradiation and no cod-liver oil. No baby receiving more than 65 minutes' total treatment up to 8 months of age developed rickets. Most of those developing rickets had intervals of two or more months without treatment.

It was found that 11 minutes' total irradiation a month without any other antirachitic measures prevented rickets in 98 per cent. of the babies studied up to 8 months of age. A smaller amount than this was found to be efficacious in many cases, particularly when the treatments were given regularly and the lapses between treatments were not too long.

From such widely separated districts as Vienna, Scotland, and Cleveland comes irrefutable evidence that healing of rachitic lesions in children yields to artificial light therapy.

Wagner (1928) gives concise details of the routine curative and prophylactic treatment

of rickets at the University clinic in Vienna. Children are irradiated three times weekly, commencing with a 2 minutes' exposure, and increasing by 2 minutes until a maximum of 15 is reached. The whole body, back and front, is exposed. In the summer solar irradiation is practised. In all cases the system has proved of great value. In Scotland, Banks (1926) gives an account of the incidence and treatment of rickets in the industrial area of Motherwell and Wishaw, in which the regular use of ultra-violet irradiation produced extremely satisfactory results. Rapid calcification in active rickets within a week has been observed by Vollmer (1926, 1927) by means of ultra-violet irradiation, combined with eosin sensitisation. It is pointed out, however, that relapses among the cases treated with ultra-violet light have occurred, and the suggestion is made that the antirachitic effect of ultra-violet irradiation is not so permanent as that of giving antirachitic vitamin.

Gerstenberger and Hartman (1929) have made a special study of the optimum dose of ultra-violet rays, as produced by the quartz lamp which, they state, with exposures given once weekly, will cure rickets within the customary time period. Using the method of Rost and Keller, they found that it required 10 minutes at a distance of 80 cm. to produce an erythema unit.

They then administered once each week an erythema unit dose to the front and back of the child, or a net total exposure of 20 minutes at 80 cm. The efficacy was not decreased in fair-skinned children by reducing the exposure to one-half the erythema unit dose, and the results were uniformly good. Zoelch (1929) states that irradiation is undoubtedly a valuable method of treatment, particularly where there is associated anæmia, debility, or infection, but points out the practical and financial difficulty of its administration over a period of several months.

These observations in children have been confirmed in numerous experiments on animals, amongst which monkeys are found to be as susceptible as rats and puppies. Hume and Smith (1927) report that even when monkeys in captivity have developed severe rickets, easily demonstrable by radiography, it can be cured by daily exposures to a mercury-vapour quartz lamp for 10 minutes.

Irradiation of nursing mothers has also been used as a prophylactic agent against rickets in the child by Hess, Weinstock, and Sherman (1927). They found a marked increase in the antirachitic potency of the milk after irradiation, and fractionisation of the milk showed that this effect was due to an augmentation of the antirachitic (unsaponifiable) factor. They suggest that such irradiation may protect nursing women from excessive drain of calcium and phosphorus.

BLOOD CALCIUM AND PHOSPHATE IN ULTRA-VIOLET IRRADIATION.—As in the case of sunlight treatment, ultra-violet irradiation has been stated to produce an almost constant rise of the lowered inorganic blood phosphate and calcium (Hess and Gutman, 1922; Howland and Kramer, 1921; Orr and co-workers, 1923; Frontali, 1929, etc.).

It has been shown by Russell, Massingale, and Howard (1928) that the amount of calcium and phosphorus in the blood varies directly with the length of exposure to the ultra-violet rays. Their values fluctuate with the curves of percentage of the bone-ash, suggesting a relationship to mineral deposition in the bones.

On the other hand, Hart, Tourtellotte, and Heyl (1928) have obtained contradictory results in human beings. They have found that an adult male upon an acidotic, calcium-deficient diet shows no increased tendency to calcium, phosphorus, or magnesium retention as a result of a daily radiation for 20 days, nor, it may be noted, as the result of 12 c.c. daily of cod-liver oil.

In cows, exposure to ultra-violet light appears to have no influence upon the calcium and phosphorus metabolism. Hart, Steenbock, and Scott (1927) irradiated pure-bred Holstein cows for 4 weeks for 1 hour daily without any effect upon the Ca or P content of the milk.

The question of the importance of ultra-violet irradiation is closely related to diet, rate of growth, and pigmentation of the skin. Hess (1924) has shown by experiments on rats that if the diet is markedly productive of rickets long exposures to light are necessary to afford protection, whereas if the diet is less inadequate short exposures suffice. Various gradations of these two factors can be formulated at will. The same is true in relation to growth. On the standard diet, a standard degree of irradiation of 4 minutes daily with the carbon arc at a distance of 3 feet will invariably protect against rickets. On this diet rats grow poorly. When it is amplified so as to lead to progressive and moderate growth the same degree of irradiation will fail to protect. Correlating these animal experiments with the same condition in human infants, Hess suggests that the atrophic or marasmic infant—notably insusceptible to rickets—has, in relation to this disorder, a far smaller requirement of active light rays than the rapidly growing normal infant, and that this may be an explanation of the “spontaneous cure” which occur in infants during the second year of life, when the growth impulse is less intense.

The effect of the pigmentation of the skin has been investigated by Hess in animals and by Levinsohn (1927) in children.

Hess showed that when two groups of rats (the melanotic form of the Norway rat) were given the minimal protective dose of light, it will be found that although diet and rate of growth were the same, the black rats developed rickets, whereas the white rats showed no rachitic lesions. He suggested that the protective rays were rendered inert by the pigment of the integument and of the fur.

In Levinsohn's experience, however, pigmented skin does not appear to have a retarding effect on the curative action of the ultra-violet rays in rickets. In an advanced case of a negro child, healing began in 3 weeks, and the child was cured in 6 weeks.

Levinsohn suggests that the ultra-violet rays do not have to penetrate the pigment in order to activate the cholesterol in the epidermal cells, and that this antirachitic cholesterol can be absorbed by the capillaries of the skin to exert its systemic effect.

(B) ADMINISTRATION OF COD-LIVER OIL.—The earlier workers, such as Trousseau and Bretonneau (1850), Freund (1905), Schabad (1909, 1910), Schloss (1913), and Orgler (1912), were convinced of the specific effect of cod-liver oil in the treatment of rickets, but in their investigations there was no suggestion that its action was due to a vitamin. The practice of Kassowitz (1884) was to suspend phosphorus in cod-liver oil, and in his opinion it was to the phosphorus rather than to the cod-liver oil that the good effects produced were to be attributed. It was not until 1919, when Mellanby's classical researches brought forward evidence of the undoubted efficacy of cod-liver oil in rickets, that its specific effect was universally recognised.

(1) Prophylactic Action.—Chick and her co-workers in Vienna (1923), Eliot in New Haven (1925), Atkinson and co-workers (1926), and Paterson and Darby in London (1925), have all carried out investigations from which the fact emerges that at any rate moderate and severe cases of rickets can be virtually eliminated by the routine administration of cod-liver oil to young children, particularly when combined with exposure to sunlight. In premature infants also, which are considered to be particularly prone to develop rickets, the disease has been shown by Gerstenberger and Nourse (1926) to be preventable by an average daily intake of 1.76 c.c. of cod-liver oil.

(2) Prenatal Prophylaxis.—The effect of prenatal administration of cod-liver oil seems to be not quite so definite, especially in the human young. Grant and Goettsch (1926) were able to prevent the severe rickets which occur in young rats of the fourth and fifth litters when the mother's reserve of antirachitic vitamin has been depleted, by adding cod-liver oil to the mother's diet. Van Leersum (1928) also found that young rats, even when subjected

to a rachitogenic diet, failed to develop rickets when the mothers had been fed during pregnancy and lactation with weekly amounts of 1.5 to 2.0 grms. of cod-liver oil.

Weech (1927) has attempted to ascertain whether the general principle of conferring on young animals some degree of protection against rickets is applicable to the human species. His results indicate that, in negro infants at any rate, rickets could not be entirely prevented by giving cod-liver oil to the mothers, but the severity of the disease seemed to be definitely lessened, and, in general, the larger the quantity of oil taken by the mother, the slighter were the evidences of rickets in her infant.

(3) **Curative Effect.**—The net result of many observations seems to be that the combination of cod-liver oil with either exposure to sunlight or ultra-violet radiation definitely produces healing of rachitic lesions. In some cases, such as those quoted by Bloomberg (1927), large doses (2 drams three times a day) had to be given to promote complete healing, but with careful consideration of the amount of cod-liver oil and the amount of sunshine received by each individual infant severe rickets can be prevented with certainty in the majority of cases.

As early as 1921, Park and Howland, on the evidence of radiographic examinations, stated that they looked upon cod-liver oil as a specific for rickets. After treating some 50 severe cases of rickets with cod-liver oil alone, keeping them as far as possible on the same diet (cereals and milk) as that upon which the rickets developed, they came definitely to the conclusion that cod-liver oil brought about a change in the bones which, if the diet were not too faulty, amounted to a complete cure. The change was not noticeable at once, but was readily demonstrable in almost all cases by the end of a month. In two or three months so much infiltration of salts had taken place that the extremities of the bones, except for deformities, were practically normal, and only differences of the finer architecture of the ends of the bones indicated the previous existence of a rachitic process.

The experiments of Webster and Hill (1929) show that the administration of cod-liver oil greatly increases the absorption of calcium and phosphorus from the intestinal tract, and that this is followed by an increased retention in the skeletal structures as shown by bone analysis.

Adams and McCollum (1928) have also attempted to correlate the effects of cod-liver oil with changes in blood and bone composition. They established the fact that cod-liver oils vary with respect to their content of vitamin D, and that both the "line test" and the ion product value $[(Ca^{++})^3 \times (PO_4^{=})^2]$ vary with the potency of the particular sample of oil given. Should a sample of oil fail to heal at a 0.125 level, progressive increase of dosage would establish a level at which healing would occur, thus giving a rough idea of the relative potency of the oil.

(4) **Cod-liver Oil Concentrates.**—Wilkins and Kramer (1927) have prepared a cod-liver oil concentrate for the treatment of rickets. The cod-liver oil was saponified and extracted with petroleum ether, and the extract evaporated, redissolved in ether, and injected intramuscularly into two children suffering from acute rickets. Fresh calcification of the rachitic cartilages appeared within three weeks although the children were being fed on a dietary which would not in itself promote calcification. On the basis of these facts, Wilkins and Kramer suggest that the antirachitic effect of cod-liver oil cannot be due to a local action upon the intestinal mucosa, but must be exerted through the circulation.

The addition of the unsaponifiable fraction of cod-liver oil to the basal ration was found by Daniels and Brooks (1927) to produce better growth in rats when it was previously mixed with corn oil than when it was mixed with water. These results fall into line with those reported by Steenbock, who found that the calcium retention of goats was not influenced by giving them cod-liver oil concentrates, unless these were previously dissolved in oil.

Rekling (1926) also found it impossible to prevent rickets produced by McCollum's diet 3143 in young rats by the use of the water-soluble constituents of cod-liver oil.

A convenient way of administering cod-liver oil so that overfeeding is avoided is described by Irish (1928). The following formula is fed in 5-ounce quantities five times in 24 hours with good results :

Milk skimmed, boiled 1 minute and cooled ($1\frac{1}{2}$ oz. to lb.)	...	15 oz.
Cod-liver oil, 3 per cent. of the quantity of milk 0.45-0.50	„
Cane sugar (add $\frac{1}{10}$ oz. per lb. of body weight)	1 „
Oatmeal water (9 oz.) <i>q. s. ad</i>	25 „

(5) **Interrupted Administration of Cod-liver Oil.**—Lewin (1927) reports an interesting case of the effect of interrupted administration of cod-liver oil on the radiographic appearance of a rachitic joint.

An X-ray photograph of the knee of a child, aged $2\frac{1}{2}$ years, with genu valgum, presented the appearance of an epiphysis within an epiphysis, in both the tibial and femoral epiphyses. The history of the child recorded an administration of cod-liver oil from the fourth to the twenty-first months, when it was discontinued for 3 months, and then resumed. It is thought that this appearance of the epiphysis was due to a two-stage growth.

(6) **Irradiated Cod-liver Oil.**—Irradiation of cod-liver oil before administration appears to have no beneficial effect on its antirachitic potency. Wyman and co-workers (1926) report that exposure of samples of cod-liver oil of known origin to a mercury-vapour lamp at a distance of about 12 inches for periods of half an hour, one and two hours made no difference to its effect on rachitic rats. The same conclusion was reached by Daniels and Brooks (1927), cod-liver oil which had been irradiated with a mercury-vapour lamp at a distance of 2 feet for 20 minutes being no more effective than the untreated oil.

According to van Leersum (1930), not only does irradiation render cod-liver oil no more effective, but it appears to become less active than the non-irradiated oil. His statement is supported by radiographic evidence and by microscopic examination of epiphysial lines.

(7) **Toxic Effects of Cod-liver Oil.**—The effect of administration of large doses of cod-liver oil has been recently correlated with the known toxicity of large doses of ergosterol. The problem has been closely studied by many workers beginning with Agduhr (1925-1926). Henriksen's experiments (1929) lead him to the conclusion that general cell degeneration throughout the body may be produced by large doses—over 1.4 gram of cod-liver oil per kilogram of body weight. Agduhr (1929) has extended his early observations on the lesions found in the hearts of certain animals given toxic doses of cod-liver oil, and has studied, in conjunction with Stenström, the appearance of the electrocardiogram in such lesions.

At first, as a result of studying some 70 mice, it was proved that even highly pronounced morphological heart injuries could be present without the electrocardiogram showing any obvious changes from what at this time could be regarded as the normal. When animals were studied more exactly over a longer period of time certain definite changes were observed, especially in the ventricular part of the electrocardiogram, which altered its features so consistently that the experimenters were able to recognise it as “a typical cod-liver oil electrocardiogram.” In addition, the auriculo-ventricular conduction time was prolonged in animals treated with cod-liver oil, in many instances to a pathological degree. The exact mechanism of these disturbances of the cardiac contraction appears to be uncertain, but there is a definite correlation with the morphological changes observed in the cardiac muscle. In less than one year, mice showed alterations in the electrocardiogram with doses of even such small amounts as 1 to 7 c.c. of cod-liver oil per kilogram of body weight daily. This would correspond bulk for bulk with approximately 25 minims of cod-liver oil daily for a 12-months-old infant, but the whole question of differences of susceptibility has to be taken into account.

C. W. Herlitz, I. Jundell, and F. Wahlgren (1929) contribute yet another paper on this subject, with reference to the harm done—more especially to the heart—by the antirachitic substance. They conducted a series of extensive and elaborate experiments on animals, and showed that doses of this substance, quite comparable with those employed in clinical medicine for children, could produce considerable degenerative changes in the cardiac muscle.

Malmberg's (1928) observations on the effect of large doses of cod-liver oil on human beings are suggestive but not indubitable.

In 2 cases of premature infants, who had received cod-liver oil during the last two weeks of life, Malmberg found atrophy and degeneration of the cardiac muscle cells, changes which agreed strikingly with those reported by Agduhr in experimental animals. In both cases the post-mortem appearances might have been attributed either to a terminal infection or to prematurity, or even to a keratosis produced by excessive fat feeding, but the interpretation of Malmberg's results must remain in doubt until each of the constituents of cod-liver oil have been tested separately.

(C) **ADMINISTRATION OF IRRADIATED FOODSTUFFS.**—In 1924 Steenbock and Black, and almost simultaneously, Hess, working on the effects of ultra-violet irradiation, found that the irradiation of many foodstuffs conferred on them the property of promoting growth and good bone calcification. A little later, Cowell, applying these findings to cases of human rickets, found that they could be cured by giving them milk which had been previously irradiated.

The treatment of rickets by "indirect heliotherapy," or the irradiation of various foodstuffs, is now regarded by some authorities as equivalent to or even exceeding in value the other forms of treatment by complete irradiation of the subject or cod-liver oil.

Daniels and co-workers (1926, 1929), for instance, have found cod-liver oil, in the amounts used in their investigations, less effective as a means of supplying vitamin D than either irradiated olive oil or irradiated milk. Daniels (1929) is unable to explain this superiority except by the suggestion that the milk and oil were carefully standardised, and that perhaps the cod-liver oil was not administered in sufficiently large doses.

In Germany and Austria particularly there is great enthusiasm for this method of treatment of rickets, which seems to be gaining ground also in America, particularly since Steenbock (1924) has patented the process of irradiating foods and medicinals, for the benefit of the Wisconsin Alumni Research Foundation. The foods which have been successfully irradiated comprise a long list, including milk, oils and fats, cereals, vegetables, orange juice, etc.

While the majority of workers are enthusiastic over the benefits conferred by the various irradiated foodstuffs, there are others like Hess and co-workers (1929), Windaus (1930), Reerink and van Wyk (1929), and Bordas and Renault (1930), who realise that it is as necessary to standardise these products as irradiated ergosterol itself.

The investigations of Reerink and van Wyk, and of Windaus particularly, show that certain substances may be formed during the process of irradiation which may have toxic effects. The methods of irradiation should therefore be selected with a view to ensuring constant and uniform products. Hess (1930) suggests the employment of filters which intercept most of the radiations shorter than 280 and 290 $\mu\mu$.

1. **Milk.**—Since 1925 many workers have reported on the efficacy of irradiated milk, both fresh and dried.

FRESH MILK.—In the prophylaxis of rickets, Scheer (1929) and Schonen (1929) have found it of great value. In Scheer's cases the prophylactic treatment of premature infants with 10 to 25 grms. daily was carried out with great success.

In active rickets the results have been equally satisfactory. Kramer showed in 1925 that liquid milk which had been exposed for 1 hour to ultra-violet light would cure severe

infantile rickets, while Degkwitz (1928, 1929) and Wieland (1928) have been specially impressed by its value, the rapidity of improvement, as shown by the disappearance of symptoms, and by alteration of the radiogram, being very striking.

Scheer (1928) states that with a daily quantity of from 300 to 600 c.c. of irradiated milk florid rickets was cured in from 4 to 5 weeks without other treatment, and Sobel and Claman (1929) report very rapid calcification of the ends of the long bones, often beginning in 2 weeks and becoming marked in 4.

The clinical value of irradiated milk has also been shown by Watson and Findlay (1929). In rickets, between 2 and 5 years of age, excellent results have followed its use when the milk has been used in strengths varying from 1 part irradiated to 2 parts unirradiated, up to equal parts of the two preparations. These workers also state that irradiated milk may prove of great value in the treatment of certain disorders other than rickets—notably disorders incidental to pregnancy and lactation, the climacteric, malnutrition, injuries, and surgical diseases of bone, and certain forms of tuberculosis.

Craniotabes, according to Sobel and Claman (1929), is not uncommonly cured in 2 weeks, even in severe cases.

DRIED MILK.—Dried milk can, according to Supplee and Dow (1927), be irradiated with no impairment of keeping quality and no development of disagreeable odour or flavour by ensuring that any given particle of milk solids remain within the activating influence of the rays for approximately 1 minute. Such a product was found superior to non-irradiated dried milk both in calcifying and growth-promoting powers.

Wieland (1928) finds that for this purpose dried milk powder, spread in a thin (3 to 4 mm.) layer, and exposed to ultra-violet light for $\frac{1}{2}$ to $\frac{3}{4}$ hour, forms a satisfactory medium.

Amongst many other workers, including Hottinger (1927), Supplee and Dow (1927), etc., Hess and his associates (1929) speak favourably of irradiated dried milk. They state that irradiated dried milk has, from the outset, seemed to be a valuable preparation. It has a definite antirachitic property which is not lost by ageing, nor is its content of vitamins A and C essentially diminished during the course of activation. This product is of moderate potency and is suited for preventive rather than for curative purposes; it is not adequate in itself to protect premature infants.

PASTEURISED MILK.—Good results with irradiated pasteurised milk are reported by Hannemann (1928).

(a) **Comparison of Irradiated Milk with other Therapeutic Agents.**—**WITH IRRADIATED ERGOSTEROL.**—Many workers, in view of the mass of evidence of the toxicity of irradiated ergosterol and its various preparations, prefer irradiated milk as a safer method of treatment. Scheer (1928), Bratusch-Marrian and Siegl (1929), and others, point out that with irradiated milk it is impossible to give an overdose of vitamin D to a child, while with irradiated ergosterol it is not only possible but dangerous.

WITH COD-LIVER OIL.—A comparison has been drawn by Coward (1929) between the potency of irradiated milk and that of cod-liver oil to the advantage of the latter. She points out that the potency of irradiated milk, which appears to be readily accessible to the public, contains 0.2 units of vitamin D per gram, as compared with an average sample of cod-liver oil containing 100 units per gram. Thus a child receiving half a teaspoonful (*i.e.* 1.6 gram) of cod-liver oil per day would receive 160 units of vitamin D. To obtain this amount of vitamin D by means of irradiated milk of potency 0.2 gram per day, he would have to receive 1.3 pints of milk.

Findlay and Watson (1929), however, do not give unqualified assent to this quantitative comparison. In their experiments, milk which, according to Coward's estimation, contained only 0.1 unit of antirachitic vitamin, gave remarkably good clinical results. Watson puts

forward, on the basis of these anomalous results, a tentative but interesting suggestion that the whole present conception of vitamin D as a purely antirachitic agent may later be found to need revision.

(b) **Methods of Irradiating Milk.**—When the irradiation of milk came to be widely practised, it was found that existing methods had two disadvantages:

- (1) Development of physical and chemical changes.
- (2) Destruction of the vitamin A potency of the milk.

(1) **PHYSICAL AND CHEMICAL CHANGES.**—There is considerable evidence that the change which takes place, giving the milk a taste and flavour which renders it unsuitable for general consumption, is due to a rancidity resembling that which occurs in commercial fats and oils by exposure to light, heat, and oxidation. The formation of nitrites has also been found to occur. Various methods have been devised to overcome the difficulty.

(a) *The Scholl System.*—The essential factor in this process is the employment of an atmosphere of carbon dioxide during irradiation. The method is described by Rietschel, Szego, and Prinke (1928), as follows: "The milk is forced under compressed carbon dioxide through three 'cells,' each having a depth of 1 mm. ($\frac{1}{8}$ th inch), and covered by a plate of rock crystal. The milk flows slowly through the cells and is thus exposed to intensive irradiation by the quartz lamp. The apparatus is water-cooled in order to prevent heating of the milk."

An alternative method of irradiation has been discussed by Gillern and Hussa (1927), the apparatus consisting of a cylinder of gauze surrounding a long mercury-vapour tube; milk passes down the cylinder in fine droplets and is irradiated as it falls, thus being activated without appreciable change of flavour.

(b) *The Scheidt System.*—In the Hoffman or Scheidt system the source of ultra-violet light is a tube containing argon as well as mercury vapour—a modification of the Scholl system which is considered by some workers more efficient. With this process, irradiation takes place in the cold, and it is therefore spoken of as the "cold process."

(c) *Heine's Method.*—Heine (1929) has suggested a simple type of apparatus in which the cream, separated from the milk, is alone irradiated, the full milk being reconstituted at the end of the process. He states that with this method no alteration of taste takes place and also that the antiscorbutic vitamin is not destroyed.

(d) *Hickman's Method.*—Hickman (1930) points out that a disadvantage common to the Scholl and Scheidt processes is the fact that the augmentation of vitamin D in their milk after irradiation is almost negligible. He considers that the weak point in their method is the formation of a thin film of milk on the surface of the glass container, so that the bulk of the milk receives practically no irradiation. He has devised a method which he claims adds very appreciable quantities of vitamin D;—2 units, according to the Pharmaceutical Society's standard.

The milk is passed over a corrugated milk cooler, 21 inches by 22 inches, running fast at first. It is then spread with a spoon until the whole surface of the cooler is covered with a thin film of milk. A quartz mercury-vapour lamp, in a reflector specially designed to focus all the rays on the cooler, is then lowered into position 11 inches from the centre of the cooler, and the milk is run at the rate of 2 gallons in 15 minutes.

(2) **DESTRUCTION OF VITAMIN A POTENCY.**—It was pointed out by Titus and co-workers (1926) and Drummond (1927) that irradiation of milk might be extremely harmful from the standpoint of its vitamin A content. It appears that this destruction is due to prolongation of the irradiation, for later work by Supplee and Dow (1927) claims that an exposure of milk in thin films to a Hanovia lamp for a few seconds, or not more than 1 minute, about doubles the antirachitic value of the milk, and apparently does not destroy the vitamin A.

A method similar to that of Hickman, described above, is stated by Nabarro and

Hickman (1930) to obviate this destruction. They estimate that by this method a particle of milk would be, on an average, 30 seconds on the cooler, but for a great part of this time it would be on the horizontal parts of the cooler, and thus scarcely affected by the rays from the lamp.

2. Milk Fat.—A protein-free irradiated milk fat, or a highly active irradiated sterol separated from milk fat, has been found by Rohr and Schultz (1927) to have a curative effect on rickets in young children, and by Schultz (1927) in young animals of various kinds, including fowls, calves, pigs, and foxes. The milk fat, or "liposterin," of which 0.1 mgrm. daily prevents or cures rickets, is stated to contain 0.05 gram of fat and 0.00005 gram of cholesterol in 0.1 gram.

3. Other Milk Products.—Goettsche and Tolnai (1926) have investigated the effects of milk products and mixtures, using as criteria clinical, radiographic, and blood phosphorus tests. Butter-milk (600 grms.), 300 grms. of albumen milk, and 140 grms. of sugar-free "Vollmilch Moro S" after 1 hour of treatment at 33 cm. distance produced Ca deposition in 18 to 36 days. With an irradiated milk mixture (3 per cent. flour, 5 per cent. butter, and whole milk), 140 grms. were effective, but 15 grms. not.

4. Cereals.—The results of animal experiments by Steenbock and co-workers (1929), and by Bacharach and Jephcott (1929), have given striking evidence of the potency of the irradiation of cereals.

In Steenbock's experiments, the different cereals, irradiated or non-irradiated, were fed at a level of 76 per cent. of the whole ration, while Bacharach and Jephcott gave 25 per cent. to bring about a lowering of the faecal pH.

(In the case of children it would be scarcely possible to make the ration contain more than 10 or 15 per cent. of cereal.)

The following table represents Steenbock's (1929) investigations into the ash content of bones of rats fed on irradiated and non-irradiated cereals for 5 weeks :

Cereal.				Non-irradiated.		Irradiated.
Farina	29.4	51.6
Muffets	43.2	52.7
Pettijohn's	30.9	49.2
Quaker oats	36.0	51.6
Macaroni	32.9	48.0

(Reproduced from the *Journal Amer. Med. Assocn.*, 26th October 1929.)

The cereals successfully irradiated by Steenbock and Daniels (1925) include wheat, rolled oats, corn, hominy, cream of wheat, shredded wheat biscuits, corn flakes, patent wheat flour, corn-starch, meat, milk, and egg-yolk.

"Irradiated farina," put on the market as a commercial preparation, has, however, been shown by Schultz and Zeigler (1929) to be unsuccessful in curing rickets when incorporated as 10 per cent. of a rachitic diet. No increase in blood phosphorus, no increase of the A/R ratio (the value of the ratio of the bone ash to the organic residue of the bones), and no increased calcification of the joints was found in such conditions.

Whole yellow corn and white corn irradiated for 30 minutes before grinding were found not to be enhanced in antirachitic value by Mussehl, Hill, and Rosenbaum (1926).

The data of Steenbock and co-workers (1929) on the results of these experiments show that with the feeding of irradiated cereals the percentage of ash as well as the total ash in the femurs was almost doubled, and their dried weight was likewise increased. Mellanby (1925) obtained similar results with dogs. In view of the recent discoveries that hypervitaminosis may occur following excessive vitamin D ingestion, Steenbock and co-workers (1929) have standardised the commercial irradiation of cereals. The standard adopted is that degree of activity which will allow normal bone production when fed as the sole source

of cereal in a rachitogenic diet, but not when diluted with three parts of untreated cereal. With this standard they claim to prevent change in palatability, destruction of vitamins, and all possibility of hypervitaminosis. By the feeding of commercially irradiated rolled oats, rickets was prevented in dogs and rats, and no interference in growth or in reproduction was shown in the latter over a duration of 15 months in four generations.

According to Tisdall and Brown (1930), irradiated cereals are not to be looked upon as a substitute for irradiated ergosterol or cod-liver oil, in the sense of being powerful antirachitic agents. Since rickets has been found to occur in its most severe form in infants fed on large amounts of cereals or other carbohydrates, they tend to regard most cereals as rickets-producing foods. The value of irradiation of cereals, they consider, lies in their change from rickets-producing to rickets-preventing foods.

5. **Vegetable Oils**—(a) **OLIVE OIL**.—The data of Steenbock and Daniels (1925) on the effect of irradiated olive oil correspond closely to the effect of cod-liver oil. Following the addition to the ordinary diet of two infants of 5 c.c. daily of olive oil, at first untreated, and later irradiated for 30 minutes, the calcium retention increased in 1 case from 0.047 gram per kilogram daily on the untreated oil to 0.061 gram on the treated oil, and in the second from 0.059 to 0.065 gram.

Irradiated olive oil has also been stated by Brahm and Mende (1926) to improve the balance of N, as well as of Ca, P, and Mg in rachitic infants.

(b) **PEANUT OIL**, when irradiated for not more than half an hour, according to Rondoni (1926), exerted a favourable action on growth and fixation of minerals in bone in rats.

(c) **FRESH CORN OIL**, irradiated with a quartz mercury lamp for 30 and 60 minutes, was found by Mussehl, Hill, and Rosenbaum (1926) to have greater antirachitic property than non-irradiated corn oil.

(d) **LINSEED OIL**.—Karóly (1927) reports positive results with linseed oil and olive oil.

6. **Orange Juice**.—Maslow, Shelling, and Kramer (1926) state that experimental rickets produced in young rats was rapidly cured by adding to their deficient diet orange juice which had been exposed to the rays of a mercury-vapour lamp for 3 hours at a distance of 18 inches. The concentrated commercial preparation of orange juice used in these experiments contained 0.12 per cent. of fat, and each animal received 15 c.c. per day.

7. **Yeast**.—Irradiated yeast has been found by Hess (1927) a valuable preventive of rickets in premature infants.

Kirsch (1928) has also stated that the antirachitic action was as marked after direct irradiation of the yeast (which has an ergosterol content of 0.8 per cent.) as after irradiation of the animals fed on yeast. The growth-promoting properties of the yeast were undiminished after 1 hour of irradiation. In a preparation preserved without special precautions, the antirachitic property, induced by irradiation, was markedly weakened after 7 months.

Wachtel (1929) has used a preparation called "cenomilchquell," containing irradiated yeast, for the vitamin enrichment of cows' and human milk, and states that it is extremely effective in promoting both the flow and the antirachitic value of the milk.

A recent detailed study of the antirachitic value of irradiated yeast has been made by Kon and Mayzner (1930). The tests were carried out on the children of the Municipal Home for Foundlings, Warsaw. The yeast was prepared as follows: "Pure bakers' yeast was used. It was always purchased fresh directly from the same reliable factory, brought immediately to the laboratory, and spread on paper until air-dry. It was then ground in a laboratory mill, and then in a ball-mill to an impalpable powder, and stored at room temperature in glass-stoppered jars. For activation the yeast was spread by means of a sieve in thin layers (50 grms. on an area of 52 by 34 cm.) on metal trays and exposed to the radiations of a Hanau quartz mercury-vapour lamp (180 v. 7.5 a.) for 30 minutes. At the

end of 15 minutes the layer of yeast was resifted, in order to expose a fresh surface to the action of the rays. No effort was made to exclude oxygen. After irradiation, 0.75 gram portions of the yeast were weighed out and sent to the children's home for testing." They report a disappearance of symptoms in rachitic children in the course of 6 to 8 weeks. Calcification of the cranial bones was generally the first clinical sign of improvement, cranio-tabes disappearing in the course of 2 to 4 weeks. This was followed by an increase of muscular tonus. Radiologically, advanced healing could be noticed in 1 month. The serum calcium remained normal throughout the experiment in the treated infants, though it was low in some of the controls.

The serum phosphorus (estimated by Briggs-Bell-Doisy method) rose rapidly within 10 days, reaching rather a high level (7.5 mgrms. per 100 c.c.), but no untoward symptoms were associated with this rise.

YEAST FAT.—It is stated by Hume, Smith, and Maclean (1928) that yeast fat, which was devoid of vitamin D before irradiation, became antirachitic for rats in a daily dosage of 0.005 mgrm. after irradiation.

8. Other Foodstuffs.—Karóly (1927) reported positive results from activation of wheat germ, butter fat, wheat flour, and cholesterol, the optimal effect being reached if 50 per cent. of the diet containing these foodstuffs were irradiated. He also states that tyrosine can be activated, though Kon and Moore (1927) have found it inactive in the proportion of 0.5 per cent. Karóly obtained negative results with synthetic fat, salt mixtures, reduced iron, proteins (casein, gelatin, wheat gluten), cane sugar, dextri-maltose, and liver-meal (available as a packing-house by-product). Of various other substances tested by Hess and Weinstock (1925) for activation by irradiation, positive results were obtained with etiolated yellow wheat, etiolated yellow lettuce leaves, and refined wheat flour, and negative results with chlorophyll, red blood cells, cream, the phosphatide of egg-yolk, and glycerol.

CHOCOLATE irradiated by the usual methods has been found to exert a favourable influence on animals (Ried, 1929), and on human beings (Krasso, 1929). In Krasso's cases of acute and subacute disease there was thought to be a shortening of convalescence with an increase in weight and appetite, but it was in severe chronic diseases that the most striking therapeutic effect was observed. Cases of severe cancerous or tuberculous cachexia reacted well to the treatment, the toxic symptoms being much improved. This was particularly apparent in the chronic exudative and fibrosed tuberculous cases, and in cases of lympho-granulomata. In all cases the therapeutic effect was enhanced by the simultaneous administration of iron. The irradiated chocolate is stated to have increased the hæmoglobin, red corpuscles, and colour index in cases of secondary anæmia and, like vigantol, augmented the liver effect in pernicious anæmia.

BRAIN.—Irradiated dried brain substance has been found by Hess (1927) to be of value in infant feeding as a rickets preventive. As is well known, the brain contains more cholesterol than any organ in the body. In Hess's first experiments the tissue was irradiated in the natural moist state, but later it was found of advantage to dry it by means of a fan and to subject the powdered residue to the rays of a mercury-vapour lamp. Ten milligrams daily of this material sufficed to bring about healing in rats receiving the standard low phosphorus (Sherman-Pappenheimer) diet. Irradiated brain maintains its potency for at least 5 months; a preparation that was irradiated in the spring was found to be practically unaltered when retested in the autumn. It was found that curative processes rapidly ensued when 1 gram daily of this preparation was given; the minimal dosage has not been ascertained. It may be added that this powder is tasteless and odourless, and can be mixed readily with milk.

SPINACH has been shown to acquire antirachitic potency on irradiation by Chick and Roscoe (1926), Mackay and Shaw (1925), and Hess (1925).

That such a wide variety of foods can be thus affected appears to be due to the fact that practically all naturally occurring foods contain lipoidal constituents of the nature of sterols which can carry this activation. In view of the alleged toxicity of irradiated substances, Wieland (1928) as the result of 4 years' experience with irradiated foods, advises the following rules: (1) Never use irradiated ergosterol in other diseases than rickets (which includes late or adolescent rickets, tetany, and osteomalacia). (2) Never exceed the maximum doses, *i.e.* 2 to 4 mgrms. irradiated ergosterol, 80 to 85 grms. irradiated dried milk powder or 500 c.c. irradiated fresh milk, 5 drops vigantol (3 times daily, in each case). (3) The specific treatment of rickets should not last longer than 8 to 10 weeks and should not be commenced or continued in the presence of chronic febrile infections, such as colds, chronic bronchopneumonia, secondary dyspepsias, etc.

9. External Application of Irradiated Substances.—According to Ried (1929) the properties acquired by fats on irradiation may make them useful for external application in some skin diseases. Irradiated fats have the property of affecting a photographic plate, and this property is increased if metals such as zinc or iron or their compounds are added to the fat before irradiation. If certain kinds of paper or metals are placed for some days near these irradiated fats, they themselves develop a similar property. The biological activity of these irradiated fats may be illustrated by their inhibiting effect on the growth of yeast cells.

(D) ADMINISTRATION OF IRRADIATED ERGOSTEROL.—For a short time after Hess's discovery that apparently pure cholesterol could be rendered powerfully antirachitic by irradiation, it was believed that cholesterol itself was the substance which produced rapid healing in rickets. Hess, Parsons, Hottinger, and many other American workers obtained good results with daily administration of 2 or 2.5 grms. of cholesterol irradiated with ultra-violet rays. Given in the form of a 3 per cent. suspension in olive oil, Hess and co-workers (1925) found cholesterol to bring about a rapid calcification of the epiphysis as well as an increase in the percentage of inorganic phosphorus in the blood. Fabre and Simonnet (1926) confirmed these results, using a 2 per cent. solution of cholesterol in petroleum ether, exposing it to a mercury-vapour lamp running at 115 volts and 3.5 amperes for periods of 15, 45, and 60 minutes. The distance of the solution from the lamp was 30 cm. After irradiation the cholesterol was suspended as a 2 per cent. solution in olive oil, and the ether driven off in a vacuum. Of this solution three drops, corresponding to 1.5 mgrm. of cholesterol, were given daily to each of the experimental rats. The optimum results were obtained with cholesterol which had been irradiated for 15 minutes.

Hess (1925) showed a threefold increase in the potency of cod-liver oil by the addition of 1 per cent. of irradiated cholesterol.

In 1929 Vitelli stated, on the contrary, that not only did irradiated cholesterol produce no good effect on the rachitic lesions produced by deficiency diet in rats, but that continual dosage was injurious. The only positive effect he observed was a slight increase in the blood phosphorus content.

Investigations by other workers, chiefly Rosenheim and Webster, after the establishment of ergosterol as the specific preserver of vitamin D, showed that perfectly pure cholesterol could not be made antirachitic by irradiation, and that whatever good effects had been attributed to its administration had been due to the associated presence of ergosterol. Irradiated ergosterol is now recognised as a definite curative agent in clinical cases of rickets, tetany, and osteomalacia. In the two latter conditions, Strote (1928) reports that severe cases which have resisted dietetic and light treatment have yielded to its administration.

The rapidity of its action in rickets is incontestable. In dogs, Hottinger (1927) produced healing in 3 to 4 weeks by a dosage of 0.1 to 1 mgrm. daily, while Hess (1930) states that "beading of the ribs has become less marked, calcification of the epiphyses evident within

a fortnight, and craniotabes has disappeared almost miraculously." Wilkes and co-workers (1929) also found craniotabes to be healed within an average of 1 month. The inorganic calcium and phosphorus rise to their normal level. Ghirardi (1929) suggests that the beneficial effect of irradiated ergosterol in rickets may be due to the fact that it can act as a powerful calcium fixator and phosphorus regulator.

(1) **Method of Administration.**—Apart from the various commercial preparations now on the market (the most widely used form being Viosterol) (see below), irradiated ergosterol is usually given either in solution in olive oil or as a colloidal solution. A method of administering it prophylactically on a large scale has been described by Degkwitz (1929). He dissolves "vitasterin" in a reduced hard fat and mixes this with dextrin so as to form a solid mass which is dispensed in packets of 5 grms., each of which contain 100 to 200 rat units of irradiated ergosterol. Each packet forms the basis for a mash of green vegetables in which it is cooked daily. The effect of this daily administration for 4 to 5 months was observed in 91 nursing infants and young children in institutions and 66 infants in their own homes. The prophylactic experiment was successful, and the preparation is stated to keep its potency over a fairly long period, and at the same time to be palatable and simple to dose correctly.

(2) **Dosage of Irradiated Ergosterol.**—Until recently the majority of workers were unanimous in postulating a dosage of from 2 to 4 mgrms. daily over a period of 4 to 5 weeks.

The majority of ergosterols irradiated contained, until the standard was laid down by the Medical Research Council (1930), from 25,000 to 100,000 physiological units of antirachitic factor per gram, this unit being the amount necessary to protect a rat of the weight of 30 grms. against the effects of a rachitigenous diet.

Lesné, Clement, and Simon (1928) considered that a child with developing rickets should receive daily for a month about 100 units of antirachitic factor, which corresponds to 1 to 4 mgrms. of ergosterol correctly and recently irradiated; such ergosterols lose almost all their efficacy at the end of 3 months.

Later work, however, such as that of György (1929), has suggested that this dosage is probably too high. György has concluded that 1 mgrm. per day for therapeutic purposes and 0.1 to 0.5 for prophylactic may be sufficient, while Vollmer (1927) has also found the latter dose quite sufficient in prophylaxis.

Hess (1930) points out that dosage expressed in terms of milligrams must be unsatisfactory, and that the most desirable standard is that which is computed in terms of a high-grade cod-liver oil. In the United States this has been done (see Viosterol), and Hess advises an average of 8 to 10 drops of this standard for prophylactic, 15 drops for curative, use.

J. H. Hess and co-workers (1930), however, state that the dosage must vary with individual cases. In some severe cases 15 and even 20 drops did not prove adequate.

Adam (1929) recommends for the prophylaxis of rickets the use of milk to which irradiated ergosterol in oil emulsion or colloidal solution has been added in the proportion of 0.001 gram of irradiated ergosterol per litre of milk.

(3) **Viosterol.**—The Council on Pharmacy and Chemistry of the American Medical Association in 1929 adopted the name of Viosterol for irradiated ergosterol and recognised two preparations of this substance, namely, viosterol in oil 100 D (N.N.R.), having 100 times the antirachitic potency of a standard cod-liver oil; and cod-liver oil with viosterol 5 D (N.N.R.), being cod-liver oil with the addition of viosterol and having 5 times the antirachitic potency of a standard cod-liver oil. Taking the titre of 100 times the potency of a high-grade cod-liver oil, Hess and co-workers (1929) formulated the following dosage: 8 to 10 drops a day as the prophylactic dose for infants who are growing at the normal rate. Premature and exceptionally rapid-growing infants must be considered as a separate group and dosage gauged according to a different scale. They will require at least 15 drops a day, and even more

should be given if signs of rickets develop. Hottinger has drawn a distinction between premature infants, which weighed more, and those which weighed less than 2 kgs. The latter group he was unable to protect satisfactorily against rickets by means of irradiated ergosterol medication. If loss of appetite or slight diarrhoea should supervene, medication should be temporarily discontinued.

For cure, 15 drops of standardised irradiated ergosterol should be prescribed in cases of mild rickets and 20 drops for the moderate cases. Severe instances, such as are rarely met with, excepting in Italians and negroes, will require still larger amounts for a short period. This same higher dosage will probably be found necessary for cases of late rickets and for osteomalacia, which are notably refractory. When exceptionally large doses are given for prevention, it would seem of advantage to control medication by occasional estimations of both the calcium and the inorganic phosphorus content of the blood.

A 1 per cent. solution of viosterol in daily doses of from 2 to 5 mgrms. has been stated to be of great value by Zallocco (1929), who prefers it to irradiated foods. In view of their more recent experiences (1930), however, Hess and co-workers now suggest that this dosage should be increased to about 30 drops a day, or, instead of giving such a large number of drops, the potency of the viosterol solution could be increased $2\frac{1}{2}$ to 3 times, so that the dosage could still be maintained at 10 drops. They state that there is no danger in the increased dosage.

PRESENT POTENCY OF VIOSTEROL PREPARATIONS.

In view of the information given by the Wisconsin Alumni Research Foundation to the Council on Pharmacy and Chemistry, the latter have decided to increase the potency of the preparations used. Instead of viosterol in oil having 100 times the vitamin D potency of a standard cod-liver oil, they have decided that it shall have 250 times that potency, while the potency of cod-liver oil with viosterol shall be increased from 5 times the potency of a standard cod-liver oil to 10 times that potency. The name of viosterol in oil 100 D is now therefore viosterol in oil 250 D, and the name of cod-liver oil with viosterol 5 D is now cod-liver oil with viosterol 10 D.

Barnes, Brady, and James (1930) express their dosage in rat units of vitamin D, and the prophylactic dose of 10 drops daily used by de Sanctis and Craig (1930) is stated by them to represent a daily dosage of at least 3000 Oslo rat units of vitamin D. This is the dosage given on the commercial products accepted by the Wisconsin Alumni Research Foundation and by the Council on Pharmacy and Chemistry of the American Medical Association.

(4) **Failures of Irradiated Ergosterol Therapy.**—Although viosterol has to a certain extent during the last year supplanted cod-liver oil as a prophylactic and therapeutic agent in the treatment of rickets, a fairly large number of workers have recently cast doubts upon its superiority. It is pointed out by Ullrich (1929) that cases occur in older children where faulty alimentary absorption is combined with bone changes characteristic of infantile rickets. The bone changes do not respond (nor do they in renal rickets as a rule) to the usual anti-rachitic measures. This is explained by the blood findings (*e.g.* hypophosphatæmia, acidosis) which vary in a completely different manner from those in the ordinary types of rickets. It should be remembered that the etiology of rickets is complicated by many factors, and that the usual good effect of irradiated ergosterol in rat and human rickets does not mean that this is the only factor involved in rachitic bone changes. The usual prompt effect of irradiated ergosterol in infants may be much retarded in the presence of infection, thus indicating that changes in the blood chemistry have a marked modifying effect. Any factors (*e.g.* acidosis, alkalosis) which tend to alter the Ca:P ratio in the blood will interfere with bone metabolism as is seen more particularly in the rickets of renal disease and intestinal infantilism.

Barnes and co-workers (1930) report that, in a group of 57 infants given 1250 rat units

of viosterol daily, 56 per cent. developed rickets, as shown by calcium and phosphorus determinations of the blood.

De Sanctis and Craig (1930) have had a similar experience. Basing their statement on the observations of Wilson and Seldowitz (1925), Moore and Dennis (1925), Wilson (1926), Barenberg and Bloomberg (1924), De Buys (1924), and others, they consider that from 25 to 30 per cent. of infants not receiving antirachitic agents develop clinical rickets. Yet they found that in infants receiving viosterol the incidence of rickets was very little lower than in those receiving no antirachitic agent. Comparing the protective potency of viosterol with that of cod-liver oil, they came to the conclusion that viosterol given in the dosage recommended at the present time by its manufacturers is less effective in the prevention of rickets than cod-liver oil. They also state that from the foregoing observations, one of two conclusions may be drawn: Either the present recommended prophylactic dose of viosterol is too small to prevent rickets, or rickets is not due to a deficiency of vitamin D alone, and they incline to the second possibility.

Holmes and Piggott (1930) also point out that when comparing the therapeutic value of cod-liver oil and viosterol, it must be remembered that viosterol has only an antirachitic function, whereas cod-liver oil has in addition a rich content of vitamin A.

Doubt has also been thrown upon the efficacy of commercial antirachitic preparations by the experiments of Garrahan and Traversaro (1929) on craniotabes. These workers used four preparations, and gave them in the doses recommended, and in some instances for considerably longer periods than was stated to be necessary. The results were disappointing. Three only of 16 patients improved, and from the time taken there is ground for suspecting that the improvement was "spontaneous," or at least ascribable in part only, if at all, to the preparation; in 9 the condition was unchanged, and in 4 it increased even while the preparation was being taken. Chemical estimations of the phosphorus content were made in 5 cases; 2 showed a slight increase, the others no difference before and after taking the vitamin preparation for 25 days.

(5) **Idiosyncrasy to Irradiated Ergosterol.**—An idiosyncrasy to irradiated ergosterol has been described by Ochsenius (1929). The sensitiveness of a child of 5 months to irradiated ergosterol, given as a prophylactic against rickets, was so great that even the ingestion of a substance containing 0.002 gram was followed by bile-stained vomiting.

(6) **Action of Irradiated Ergosterol on Healthy Adults.**—The effects of administering irradiated ergosterol to healthy adults appear to be quite different from those obtained in the case of children with rickets.

In 1927 Kroetz carried out an experiment on 4 healthy adults. They were placed on a standard diet for 2 days and then on the experimental diet for a period of 7 to 8 days. The experimental diet consisted of the standard diet to which 18 mgrms. of Merck's irradiated ergosterol were added in the case of adults A and B, and 30 mgrms. in the case of C and D. After the experimental period of 7 to 8 days, the patients were given 3 days' rest and were then themselves irradiated. Estimations were made of the alveolar carbon dioxide, of the blood carbon dioxide, of the serum phosphorus, sodium, calcium, chlorine, carbonic acid, and inorganic phosphate, and of the urine chlorine, phosphorus, and pH. They concluded from their results that the effects of administering irradiated ergosterol to healthy adults are opposite to those found in the rachitic child. Thus a slight increase of phosphorus and a definite rise in the chlorine of the urine were found, together with an increase in the amount of water excreted. Further, there was a decrease of serum phosphorus and a recognisable blood acidosis. No parallelism could be traced between the action of ultra-violet light on the patients and the administration of vitamin D *per os*.

Havard and Hoyle (1928) carried out a similar investigation, but they used only 8 mgrms.

per day of irradiated ergosterol. They found that a dosage of this amount caused no significant change in the blood inorganic phosphate or serum Ca. Radiation for a 16-day period similarly had no effect.

These results have been more or less closely confirmed by Hart, Tourtellotte, and Heyl (1928), and by Blunt and Cowan (1929). In the experiment of Hart and co-workers a young man was put on an acidotic diet low in calcium and phosphorus, so that he was excreting both elements. Both cod-liver oil and irradiation of the whole body failed to lessen the almost constant negative balance of calcium and phosphorus.

In Blunt and Cowan's experiment, the subjects—7 normal women—attempted to produce a negative calcium balance by curtailing their calcium intake to 0.23 to 0.275 gram a day. Vitamin D administered in the form of irradiated rolled oats (proved by rat experiments to have decided antirachitic potency) failed, however, to check the excretion of calcium. Blunt and Cowan suggest that a short withdrawal of vitamin D or its addition in moderate amounts has no marked effect on the Ca and P metabolism of the adult, but that large amounts of it may possibly do harm by causing an increased excretion of Ca. Where there is some special demand for Ca or P in the adult, such as during pregnancy and lactation, vitamin D appears to have some beneficial influence. They consider that if the evidence for the stimulation of parathyroid hormone by vitamin D is accepted, it may be used to explain this difference in the action of irradiated ergosterol in young and adult animals. From this point of view the increase in calcium produced by parathyroid activity will be retained by the young, growing animal, which has a constant need for calcium, while in the case of the adult animal the excess of calcium will not be retained but actually lost, except in conditions, such as lactation, where there is an increased demand for calcium.

(7) **Commercial Preparations of Irradiated Ergosterol.**—Several preparations of ergosterol, which have been proved to have curative effects on rickets, have been placed on the market in Germany and in England. Various methods have been suggested for the estimation of their vitamin D content. Most of these are based on determinations of the minimum amounts required to produce healing in the case of experimentally produced rickets in rats.

Scheunert and Schieblich (1929) have devised a method based on the minimum amounts of the preparations necessary to add to the rickets-producing diet in order to prevent the onset of rickets. Various trade preparations were tested by this method, and it was found that vigantol contained 25,000, radiostol 2000, and präformin 800 to 1000 protective units per c.c. of the preparation.

(a) **VIGANTOL.**—Numerous workers, including Pfannenstiel (1928), Falkenheim (1927), Schippers (1928), Wiskott (1928), Liégeois (1928), Prinke (1927), Lasch (1928), Karelitz (1928), etc., have reported the rapid healing of rickets, as estimated by the rise in serum phosphorus and calcium and the X-ray examination of the bones, after administration of vigantol.

Falkenheim (1927) used three preparations—a solution in olive oil, pastilles made up with coco-butter, and sugar-coated tablets. The three preparations were rapidly effective in uncomplicated cases of rickets, but the impression was gained that the olive-oil preparation was the most satisfactory, partly because it was easily assimilated and partly perhaps because the ergosterol in this form retains its activity for longer periods without loss.

The most effective dose appears to be 2 to 4 mgrms. daily, or, as in Schippers' cases, 4 to 5 drops of a 1 per cent. solution twice daily.

Kollman (1929) has found that a dose of 2 mgrms. per day of vigantol was sufficient to bring about complete healing of rickets in all cases. The average time of healing was 26 days, being the same for children 2 years old as for those 2 to 3 months old. He advocates the use of capsules of vigantol as giving a more accurate dose than is obtained by feeding a certain number of drops.

For prophylactic purposes, smaller doses, 1 to 3 mgrms. daily, have been found, according to Vollmer (1928), Lasch (1928), Aengenendt (1928), and others, to prevent rickets in prematurely born infants and twins, and to protect them from the risks of malnutrition.

György (1929), Starlinger (1927), Hottinger (1929), and others all report that they have also obtained curative results in cases of severe osteomalacia from the daily administration of 3 to 10 mgrms. of vigantol, but only after some months of treatment.

Prinke states that a comparison of the results obtained with the irradiated ergosterol supplied by Windaus, and with those following trials with vigantol, gave the impression that the latter was not quite so potent as Windaus' original preparation.

Heubner and Holtz (1929) state that vigantol is more powerfully antirachitic than radiostol (given in equivalent quantities of ergosterol) and is also more toxic to roughly the same extent.

(i) *Skin Changes after Vigantol Treatment.*—Pigmentation of the skin in children has been observed by several observers after more or less prolonged treatment with vigantol.

In the case described by Bernheim Karrer (1929) a child was kept for 25 days on 5 mgrms., and for a further period on 10 mgrms., of vigantol per day. A month after omitting vigantol the skin was darker than that of a twin control.

In Rodecourt's (1929) cases the changes seen in the skin of three infants treated with vigantol resembled those seen after over-exposure to sunlight. In 1 case the skin was pigmented and in the others it was raw and dry. There was no evidence of any faulty manufacture of the material used, but Rodecourt remained doubtful as to whether the skin changes were accidental. However, in 11 later cases of infants treated with vigantol, 2 were found showing a yellowish-brown pigmentation of the skin. One infant, 17 days old, received 5 drops of vigantol 3 times daily for 7 days. The other, 3 months old, received 5 drops twice daily for a longer period. After the administration of the vigantol was stopped there was a relatively rapid disappearance of the skin signs. Most of the infants reacting in this manner were dark-skinned and dark-haired. The effect of sunlight could be excluded as a factor apart from the fact that the effect was observed over the whole body and not just on the exposed sites.

(ii) *Neuro-psychical Changes after Vigantol Treatment.*—A disturbance of the neuro-psychical state in infants treated otherwise successfully by vigantol has been reported by Gil (1928). The dosage of vigantol had been 0.004 gram. Gil attributes the condition to an excess of potassium in the blood and tissues as the result of the action of vigantol in transporting calcium from the blood and other tissues to the bones. He suggests that calcium and potassium should be given during vigantol therapy.

(b) *RADIOSTOL AND RADIOSTOLEUM.*—British Drug-Houses have issued a preparation of irradiated ergosterol (radiostol) in the form of a solution, each c.c. possessing a vitamin D activity equivalent to 10,000 antirachitic units, and in the form of pellets, each pellet with an activity equivalent to 6000 antirachitic units. Radiostoleum is a mixture of radiostol and a concentrate of vitamin A in a solution of oil, each c.c. containing 10,000 antirachitic units. Cowell (1928) and Aidin (1928) have reported rapid healing of bony lesions, increase in weight, and improvement in the general condition with a dosage of 15 to 30 drops daily of radiostol. Radiostoleum was given by Cowell to one child with the same clinical result.

(c) *PRÄFORMIN.*—This preparation, in doses of 2 mgrms. per day added to milk, is reported by Wiedow (1928) to have produced a rapid cure of rickets and an average gain in weight.

(d) *TABLOID IRRADIATED ERGOSTEROL.*—Burroughs, Wellcome & Co. have issued a tabloid form of irradiated ergosterol, each tabloid containing 0.15 mgrm.

XXXIII. EFFECTS OF LARGE DOSES OF IRRADIATED ERGOSTEROL.

Reference has already been made (Section IV, p. 20) to the effects of administration of large quantities of vitamin D in the form of irradiated ergosterol. There has been so much discrepancy in the results of groups of workers on this subject that even with the clear evidence now available the whole problem is not yet elucidated. It is certain that toxic effects can be produced by large doses of irradiated ergosterol; it is also certain that the dose necessary to produce such effects is very much greater—a hundred, or even a thousand times—than that which is usually given to prevent or cure rickets. So strongly has this latter fact been established that the Council on Pharmacy and Chemistry of the American Medical Association, has, as mentioned above, decided to increase the concentration of the standard viosterol in oil from 100 D to 250 D. At the same time it seems certain that a true hypervitaminosis D can occur, with a disturbance of calcium and phosphorus metabolism, and the question of overdosage and the mechanism of production of toxic effects must still be regarded as under consideration.

The opinions of workers on this subject may be grouped as follows :

- (1) Those who postulate a lethal effect of overdosage.
- (2) Those who have found definite symptoms of toxicity.
- (3) Those who have found no ill-effects even with a dosage considerably larger than that required in the treatment of rickets.

(A) **LETHAL EFFECTS.**—This group of workers is chiefly represented by the German school, including Pfannenstiel (1928) and Krietmar and Moll (1928).

Pfannenstiel (1925) found that overdosage or prolonged administration of vigantol in the rabbit led to a loss of weight, and in some cases to death, with symptoms of very severe cachexia.

He emphasises the statement that these toxic symptoms are not caused in rickety animals, and that it is only when the vitamin balance is normal that symptoms arise from giving a pure source of the vitamin.

Kreitmar and Moll (1928), working on a large scale, came to similar conclusions. White mice, which received 2 mgrms. a day of irradiated ergosterol, showed after some days a loss of appetite and activity, alopecia, somnolence, quickened respiration, loss of weight, and diarrhoea, death occurring after the sixteenth day. With a daily dose of 1 mgrm. the same phenomena showed themselves, but more slowly; some of the animals died, while others survived. With 0.5 mgrm. a day the only symptom observed was loss of weight.

The guinea-pig was found more resistant than the rat, 40 mgrms. being the fatal dose, and 10 mgrms. the toxic dose, as evidenced by loss of weight. For the rabbit, on the other hand, the fatal dose was 2 mgrms., and the toxic 0.25 mgrm.; and for the cat, 10 mgrms. fatal, and 5 mgrms. toxic. The post-mortem appearances showed great loss of fat, enteritis, and considerable atrophy of the spleen, which was pale and yellow. There was also found a generalised calcification.

Heubner and Holtz (1929) have also reported a high percentage of deaths in rabbits fed on 4 c.c. of radiostol for 13 to 29 days, and Smith and Elvove (1929) have shown that repeated administration of 2 mgrms. and upward of irradiated ergosterol given orally or intramuscularly to full-grown rabbits may prove fatal in a relatively short time. Harris and Moore (1928, 1929) and Harris and Stewart (1929) have had results practically identical with those of the German workers, with, in addition, atrophy of the thymus.

(B) **TOXIC EFFECTS**—(1) **General.**—Anorexia and loss of weight, with impairment of the

general physical condition and lack of growth, have been observed in animals by Klein (1929) though he did not confirm the lethal effect of the German workers. He also found the blood calcium concentration higher, the protein concentration of the serum lower, and the albumin-globulin ratio higher in the animals on ergosterol.

Collazo, Rubino, and Varela (1928) described a similar picture of hypervitaminosis D (5 mgrms. of vigantol) in rats. The symptoms were loss of weight up to 40 per cent., retardation of development, subnormal temperature, bristling of the hair, moisture of the skin, torpor, profound exhaustion, paresis, and diarrhoea at the end.

Dealing with the effects of large doses of irradiated ergosterol on human beings, Hess and Lewis (1928) have found ill-effects to occur only after several weeks of treatment. In certain cases excessive calcification was recorded, and 2 infants showed associated symptoms of loss of weight, with vomiting, fever, and slight drowsiness.

Bamberger's (1929) experience with children treated with large doses of vigantol have differed in some respects from those of Hess. The former did not find a supernormal blood calcium with hypercalcification of the epiphyses in his toxic cases. There was loss of weight with severe vomiting, and hyaline and granular casts, red blood cells and albumin appeared in the urine, and the serum Na and Cl increased (Na about 10 per cent. and Cl about 5 per cent). The hypertonicity of the serum induced by the vigantol treatment appeared to be associated with renal damage.

Since no harmful effects were observed with an unsaponifiable extract of cod-liver oil, though the dose of irradiated ergosterol so given was almost equal to that given in the form of vigantol, Bamberger concludes that the clinical findings in these cases cannot be associated with hypervitaminosis, and that the pathological symptoms induced by vigantol must be due to contaminating substances or decomposition products.

(2) **Calcification.**—Large doses of ergosterol indubitably bring about a more or less marked hypercalcaemia, and calcium deposits in various organs have been described by many workers. Whether this effect is due to an increased absorption or decreased elimination of calcium and phosphorus, or whether the excess of these elements in the blood is derived from stores of calcium in the body (*e.g.* in the bones), which can be mobilised by overdosage of vitamin D is as yet unknown. Ashford (1930) has shown that hypercalcaemia and hyperphosphataemia do occur, while Watchorn (1930) believes that the excess cannot be accounted for on the grounds of increased absorption and retention. An investigation carried out by Smith and Elvove (1929) showed that the calcium deposits in the tissues of the animals receiving the larger doses of irradiated ergosterol were especially pronounced in the thoracic aorta, the kidneys, and the lungs. In the latter the most frequent site for calcium deposition was the bronchial cartilage, and the less frequent the interalveolar septums. Calcium deposits were not seen in the pulmonary or renal vessels. In the kidney the calcium deposits were usually seen in the convoluted tubules of the cortex and as calcium casts in the straight uriniferous tubules. Deposition of calcium in the muscular coat of the aorta was noted with greatest frequency, and was usually more pronounced than in the other situations enumerated. There was no microscopic evidence of calcium deposits in the heart, liver, or spleen. It should be noted further that harm to the kidney in the nature of an acute or chronic diffuse nephritis usually accompanied the calcification on the larger doses of irradiated ergosterol, and in a few instances similar kidney injury was noted after the small dose of 1 mgrm., despite the complete absence of demonstrable calcific deposits. Smith and Elvove maintain that the hypercalcaemia induced is not sufficient *per se* to explain the abnormal deposits of calcium in the tissues, but that coincident high content of inorganic phosphate in the blood is essential.

These results are confirmed by Jundell (1929) who has found cardiac lesions (degenerative myocardial changes and deposits of calcium) in mice fed on large doses of ergosterol, whether

irradiated or not. Marked changes in the arteries have been observed by Wenzel (1928) and Huckel and Wenzel (1929) in rabbits which had received 50 to 70 mgrms. of viosterol, the ascending portion of the aorta being thickened and its inner surface rough, with hard plaques projecting in the lumen. In animals which had received from 100 to 200 mgrms. of viosterol the process involved the entire aorta. "In all the animals which had received still larger doses the whole aorta had been converted into a rigid tube which curled outward when incised longitudinally. The extensive, hard deposits of calcium, with intervening large and small aneurysms and the rough inner surface of the aorta, constituted a characteristic picture of the vascular injuries produced by viosterol. Compared with these changes in the aorta, the changes in the large arteries were unimportant. Microscopically, calcification could not be demonstrated in animals which had received a total dose of only 12 mgrms.; in these animals, however, marked changes in the media were noted. The outer layers of the media were loosened and presented a honeycomb marking. Large vesicular cells which did not contain fat were present. The irregularity of the inner surface of the aorta was particularly marked in one animal which had received a total of 14 mgrms. In the projections the fibres of the media were loosened. Particularly the inner layers of the media were characterised by localised areas that stained poorly; in these areas the elastic fibres were decreased in number and showed interruptions in their continuity. In some places in the aorta, structures resembling giant cells were noted, together with degeneration of the nuclei and fibres and calcification. In areas in which the calcification of the media was marked, the overlying intima had become necrosed, whereas the outer layers of the media were almost unchanged. From this study it is evident that the first morphologic changes in the development of the process occur in the media. Examination of the arteries of the kidney revealed that here, also, the pathologic process began in the media. Changes in the media of the arteries of the kidneys were noted in animals that had received only 9 mgrms. of viosterol. Contrary to the changes in the aorta, large doses of viosterol produced extensive primary necrosis of the media of the arterioles of the kidney."

Similar evidence of toxicity is brought forward by Moreau, Rubino, and co-workers (1928) and by Heubner and Holtz (1929). In experiments by the latter workers, two of three rabbits fed on 4 c.c. of radiostol showed pronounced arteriosclerosis, the wall of the arch of the aorta showing aneurysmal dilatations and numerous calcium deposits, whilst the aorta in its whole course down to the pelvis was calcified. The other rabbit showed only a small calcium deposit in the aortic arch. The control animals fed on 4 c.c. of arachis oil alone were killed after 29 days. Two were healthy whilst 1 showed very small degenerated patches in the aortic arch which, it is suggested, may have been due to the large quantity of oil taken, or to some intercurrent disease.

Calcification of the superior vena cava and liver in rabbits fed on large doses of vigantol has been reported by Fischl and Epstein (1929), and of the lungs, kidneys, and stomach in white mice by Rabl (1929).

Pathological changes in pregnant rats given large doses of irradiated ergosterol in the form of vigantol are reported by Schoenholz (1929), but the number of animals used was not sufficient for conclusive evidence. The amounts given varied from 10 to 25 drops (4 to 10 mgrms.) per day, although the actual quantities consumed were uncertain, since, owing to loss of appetite (more especially in the case of those animals fed with the larger amounts of vigantol), portions of the diet remained uneaten. Post-mortem examination of the pregnant animals revealed typical deposition of calcium salts in various organs (including the liver). In some cases, evidence was obtained of the resorption of the foetus, and, where litters were born, the young were usually small and showed retarded growth.

The following lesions are recognised by Duguid, Duggan, and Gough (1930) as indications of the toxic effects of irradiated ergosterol. They were notably more present in the

rats on a synthetic vitamin-free high calcium diet than in those on a diet of bread and potatoes :

" *Kidneys* : calcareous casts in the tubules, with considerable local destruction of the epithelium and frequently a more widespread tubular nephritis.

" *Aorta* : focal and sometimes widespread degeneration, fading or disappearance of the medial muscle, usually with calcification of the affected part.

" *Heart* : focal degeneration and calcification of the muscle fibres and occasional calcification of the walls of the coronary arteries.

" *Stomach* : scattered calcium deposits both in the mucous membrane and in the muscular coat.

" Calcium deposits were frequently found scattered in other parts of the body, but not with any degree of regularity."

That calcification is not indicative of the degree of toxicity has been stated by Levaditi and Po (1930). They found that although calcification was increased with addition of the active principle, rabbits and mice that died with symptoms of acute intoxication after large doses of viosterol did not reveal abnormal areas of calcification in the tissues or organs. On the other hand, it was found that calcification can be initiated in lesions due to bacteria or an ultravirus (as in tuberculosis or in chronic encephalitis).

(3) **Changes in the Bones and Teeth.**—The effect of large doses of vigantol on the bones and teeth has been investigated by Weinmann (1929). Rats which had received a daily dose of 0.5 c.c. of vigantol dissolved in oil showed not only cachexia but pathological calcification of the upper jaw and teeth. Certain areas of the bone and cementum were overcalcified, while the surrounding zones showed resorption of tissue. There were marked deposits of calcium in the membrane between tooth and alveolar margin resulting in a union of tooth and bone.

Wiskott (1930) also reports changes in the bones in normal rats receiving large doses of viosterol. He found that, although the weight of the animals increased normally, there was a marked deficiency in the dried substance of all the bones as compared with controls from the same litter. There was an even greater deficiency in the amount of ash and its principal constituents, calcium and phosphorus. The administration of large amounts of calcium did not prevent these changes in the composition of the bones. The calcium content of the internal organs did not correspond to the calcium deficiency in the bones. He was unable to determine whether the ash loss was caused by increased resorption or decreased calcification, but on the basis of his observations he believes that although overdosage of viosterol produces X-ray changes only in the zones of growth, it is possible that the mineral content of the entire bone is decreased.

(4) **Epithelial Overgrowth.**—A species of tumour growth, which they believe to be a symptom of hypervitaminosis D, is reported by Collazo, Varela, and Rubino (1928).

In two rabbits which died in 30 days after a daily dose of 230 mgrms. of irradiated ergosterol, the gastric mucosa was hypertrophied, and infiltration of the deeper layers was found; the pylorus and cardia were thickened. Many control animals of the same size and original weight showed no such changes. Histologically, the appearance of a true epithelioma is said to have been found: an atypical arrangement of the glandular tubes and epithelium, with invasion of the submucosa—rapid proliferation, rupture of the basal membrane, and epithelial downgrowth.

(5) **Changes in the Blood.**—A decrease in lymphocytes, and an increase of monocytes, by feeding rabbits with large overdoses of ergosterol (15 to 25 mgrms. per kg.) dissolved in olive oil, is reported by Fraser (1930). It was found that, after a preliminary rise, the lymphocytes were decreased by 30 to 50 per cent. of their initial value. On discontinuing the feeding

of irradiated ergosterol, the lymphocytes soon returned to the normal value. The polynuclear cells first decreased and then increased as the animal began to lose weight.

(6) **Cerebral Disturbances.**—It has been stated by Hoff (1930) that chronic poisoning with vitamin D leads to severe cerebral disturbances. He attributes this effect to an increased resistance of the cell membrane produced by large doses of vitamin D. He has found that the administration of such doses increases the resistance against metallic poisons, and infers that with chronic poisoning the cell membrane acquires this resistance, causing the cell to be shut off from normal nutritional substances, and therefore liable to injury.

(C) **ALLEGED RELATIVE NON-TOXICITY OF IRRADIATED ERGOSTEROL.**—In spite of the accumulating evidence of the toxicity of irradiated ergosterol, some workers have found difficulty in reproducing the lesions.

Dixon and Hoyle (1928) have found no lethal effects even with very large doses, and Hoyle and Buckland (1929) have recently confirmed their results. These latter workers observed a diuresis, without an increased excretion of phosphorus and chloride, calcium phosphate calculi in kidneys, ureters, or bladder, and some arteriosclerosis of the aorta, but no marked loss of weight, and no fatal effects.

More recently (1930), Hoyle states that the toxic effects of ergosterol which has been irradiated in alcoholic solution are produced in rats only when the animals are taking a synthetic diet. "If a natural diet of bread and milk is given, arterial disease and the other toxic features are absent. Such animals, however, fail to gain weight at the normal rate and show urinary calculi after a time if the experiment is prolonged, just as do animals receiving large doses of ergosterol that has been irradiated in oil."

Hoyle believes that the toxic action of alcohol-irradiated ergosterol is due to two factors: (1) an unknown toxic substance; (2) vitamin D, and that over-irradiation destroys first the vitamin, and later the toxic substance. He states that the formation of the toxic substance is facilitated when the ergosterol is irradiated in solutions such as alcohol, and inhibited by using oil as the solvent.

Comel (1929) also reports no ill-effects in either rachitic or normal rats from a dosage of 10 mgrms. of irradiated ergosterol daily. According to Cartland, Speer, and Heyl (1929), a dose of irradiated ergosterol 1000 times the physiological produced no demonstrable pathological effects. In their experiments three groups of three rats were placed on the following diet: whole wheat, 65; whole milk powder, 32.5; NaCl, 1; Ca lactate, 1.5. Each group was given respectively 1100 and 1000 rat units of ergosterol per rat daily for a period of 8 weeks. The diet contained Ca and P in such proportions that any possible hypermineralisation was favoured. Two sister pups received respectively 2000 and 200,000 rat units of irradiated ergosterol on a diet of whole wheat bread, milk, and meat. They were killed after 18 weeks. There was no evidence of toxic effects in either rats or dogs, growth proceeding normally. In none of the rats was there any increase in Ca or P retention nor post-mortem evidence of hypercalcification. The Ca and P content of the blood serum of the dogs was normal, whilst X-ray examination at the end of the ninth week showed no hypercalcification of the bones or calcification in the soft tissues. At autopsy the dogs showed no pathological evidence of hypercalcification, and the Ca content of the aorta was the same in each dog. Tooth development was normal in both rats and dogs.

Investigating the effects on the bones, Kramer, Shear, and Mackenzie (1929) have found very little actual difference in the composition. The bones of young rats which had been fed massive doses of irradiated ergosterol for 1 month were analysed for Ca, inorganic phosphate, and CO₂. From these data the ratio residual Ca: P was calculated. The ratios obtained for the bones of the rats which had been fed irradiated ergosterol were

the same as those obtained for the bones of control rats and of rats which had been fed cod-liver oil.

(D) **TOXICITY WITH EXTREMELY LARGE OVERDOSAGE.**—An overdosage of 1000 times was found just perceptibly harmful by Bills and Wirick (1930), 4000 times overdosage definitely injurious, and 40,000 times overdosage strongly toxic. The toxic action was increased by calcium carbonate, and was unaffected or slightly lessened by disodium phosphate.

(E) **SUGGESTED EXPLANATIONS OF DISCREPANT RESULTS**—(1) **An Independent Toxic Factor.**—Dixon and Hoyle (1928) explain the discrepancy in the various results by suggesting that some independent toxic factor may have been at work, possibly the solvent used by the German workers in irradiating the ergosterol. This question of associated toxic products, independent of the properties of ergosterol itself, is one which is still under discussion. While Seel (1928) and Reyher and Walkhoff (1928) believe that irradiation produces the toxic substances, and Aghdur (1926–1928) that several substances in cod-liver oil combine to produce toxic effects, Bills and co-workers (1930) have found it possible to produce an irradiated ergosterol that is remarkably non-toxic, more than 4000 times the therapeutic dose of such a preparation being required to produce a hypercalcification.

Reyher and Walkhoff suggest that the irradiation may produce nitrites in food substances, but they do not explain how nitrites could be produced by irradiation from pure ergosterol.

Borghi (1929), on the other hand, like Dixon and Hoyle, believes that the toxic effects produced by other workers, which he has failed to confirm, are due, not to vitamin D itself, but to some impurity associated with it. He bases his assumption on an interesting experiment which he has carried out on splenectomised rats, following Schmidtman's observation that the deposition of calcium in various organs is greater in splenectomised than in normal animals.

Schmidtman (1928) deduced from his experiment that the spleen has strongly the property of absorbing an excess of ergosterol. Borghi, however, using as his source of ergosterol a preparation called "Vitadol," standardised to contain 10 mgrms. of vitamin D in 1 c.c., in a dosage of 1 c.c. subcutaneously on alternate days, has failed to find any deposition of calcium salts either in the normal or the splenectomised animals, though he found a certain amount of fatty degeneration of the liver and kidneys.

Perhaps the clearest evidence in support of the theory that irradiated ergosterol can produce a specific hypervitaminosis, quite apart from any toxic products which may be formed extraneously, is that recently brought forward by Harris and Moore (1929). They have found that over-irradiated and non-irradiated ergosterol are both non-toxic—a fact which seems to show that it is the vitamin D produced by the optimum amount of irradiation which, when given in excessive doses, has injurious effects.

It must be stated, however, that recent experiments by Simonnet and Tanret (1929) do not confirm this view. They found that whereas ergosterol irradiated for a short time produced no toxic effects in mice when fed at the rate of 5000 times the maximum dose, the same dosage of ergosterol, irradiated for 6 hours, produced congestive lesions of the intestinal tract leading to death. Since no evidence of hypercalcification was found, and some of the animals killed after 4 weeks were apparently normal, even after receiving the ergosterol which had been irradiated for a long time, these observations can scarcely be regarded as conclusive.

(2) **Different Species of Animals.**—It is possible that some of the conflicting results may be reconciled by the suggestion of Jacqmin and Ledecq (1929) that different species of animals react differently to an excess of vitamin D. In rabbits, for instance, with a dosage of 15 to

20 mgrms. daily of irradiated ergosterol, they found very definite evidence of generalised calcification, especially in the aorta and other blood vessels. In mice, on the other hand, a daily dosage of 10 mgrms. produced no characteristic lesions. Similarly Levaditi and Po (1930) state that monkeys appear to be less susceptible than other laboratory animals to the bad effects of overdosage.

(3) **Vitamin Imbalance.**—Another and equally possible hypothesis is that of “vitamin imbalance,” *i.e.* that overdosage of vitamin D may be more injurious when applied in conjunction with deficiency of another vitamin. This theory has been discussed above.

Höjer (1926), for instance, showed that large doses of cod-liver oil, when given with a diet insufficient in vitamin B, produced focal necrosis of cardiac muscle. Harris and Moore (1928) reported a similar experience in that they found that a high marmite diet gave increased tolerance for a vitamin A and D concentrate in the form of cod-liver oil. They found, however, that irradiated ergosterol lesions were not modified by giving large amounts of marmite, and they, like Höjer, were of the opinion that vitamins A and B were the opposing or balancing vitamins concerned in the cod-liver oil experiments.

(4) **Calcium Content of the Diet.**—A theory has recently been advanced that irradiated ergosterol may be more toxic to animals if their vitamin-free basal diet has a high calcium content.

It has been shown by Schmidtman (1928) and Herzenberg (1929) that a high calcium diet favours the early production of vitamin D lesions, and Rabl (1929) has confirmed these observations by his finding that the addition of calcium salts to a potato diet produces signs of hypervitaminosis D. Harris (1930) has also stated that calcification on a vitamin-free diet depends not only upon the degree of vitamin D excess, but also upon its calcium and phosphate content. On the basis of these facts, Duguid, Duggan, and Gough (1930) have carried out experiments with a vitamin-free diet notably high in calcium content (0.6 per cent.) which have led them to conclude that in preparing a synthetic diet for use in investigating the toxicity of irradiated ergosterol, attention must be paid to the calcium content of the diet.

(F) **MECHANISM OF HYPERVITAMINOSIS D** — (1) **Hypercalcaemia.** — According to Hess the outstanding manifestation of the toxic reaction is hypercalcaemia. In rats, which had received large doses of irradiated ergosterol, concentrations of 18 to 20 mgrms. per 100 c.c. of serum have been found instead of the normal 10 to 11 mgrms., and a rapidly-growing premature infant showed a concentration of 13.6 mgrms. Hess and co-workers, together with Greenwald and Gross, incline to the view that the mechanism of this condition lies in an over-stimulation by the irradiated ergosterol of the parathyroid or other glands which stimulate calcium metabolism. They suggest that the excessive deposition of calcium in the tissues so produced indicates a greatly heightened assimilation of calcium, that it is maintained not by increased absorption but by mobilisation from depots within the body, and that it is accompanied by increased excretion and by excessive loss of calcium from the bones.

In this connection it is of interest to note the recent work of Bauer, Aub, and Allbright (1929) on the mineral reserve of the body. They have shown that in animals on a low calcium diet the bone trabeculae of the ends of the forelegs tend to disappear, while new trabeculae are formed during a high calcium diet. Greenwald and Gross's theory (1925, 1928) of mobile depots within the body seems to coincide with the view of a labile reserve of calcium in the bone trabeculae. Their conclusions are supported by a series of animal experiments in which the ash of the bones was determined after 1 mgrm. or more of a highly active ergosterol had

been given. In one instance, whereas the bones of the control rats contained about 52 per cent. of ash, those which had received 1 mgrm. of the German irradiated ergosterol preparation for a period of 14 days contained only 48 per cent. ; in a similar test, in which as much as 5 mgrms. daily of this preparation had been given, the bones of the control animals contained about 43 per cent. of ash compared to only 39 per cent. in the treated animals. Other tests confirm the conclusion that the giving of excessive amounts of irradiated ergosterol may lead to a withdrawal of calcium and phosphorus from the bones, especially if the diet is deficient in these constituents. These results complement the metabolism experiments of Hottinger (1929) who found that very large amounts of irradiated ergosterol, when given to puppies, induced negative instead of positive balances of calcium and phosphorus. It should be added that excessive amounts of cod-liver oil may likewise lead to a withdrawal of inorganic salts from the skeleton.

(2) **Hyperphosphatæmia.**—A hyperphosphatæmia has also been shown to occur a few weeks after irradiated ergosterol has been given. In Hess's experiments (1928) the increased concentration of phosphate ranged only from 8 to 10 mgrms. per 100 c.c. of blood, and he believed it to have no significance other than that of a stabilising action on the part of the body. More recent experiments by Harris and Stuart (1929), however, have shown that a dosage of irradiated ergosterol of 0.1 per cent. produces in young rats a 50 per cent. increase of phosphate, with a 25 per cent. increase of calcium, while in adult rats 10 mgrms. per day produced a 50 per cent. increase of phosphate, with no rise of calcium, but with abnormal deposits in the body.

Hess believes that the induction of an excess of calcium in the blood is not distinctive of irradiated ergosterol, but is simply a sign of overdosage of an antirachitic agent, and may result from giving large amounts of cod-liver oil. The reason hypercalcæmia rarely develops in infants as a result of cod-liver oil medication is simply that one cannot give more than about from 6 to 8 teaspoonfuls daily. It has been suggested that irradiated ergosterol causes hypercalcæmia because it is dissociated from the cholesterol and fat with which it is bound in nature. This suggestion is untenable, in view of the fact that large amounts of cod-liver oil do produce hypercalcæmia, since ergosterol in cod-liver oil is intimately associated both with cholesterol and fat.

The theory of Harris and Moore (1929), with regard to the mechanism of a specific hypervitaminosis, would include both the hypercalcæmia and the hyperphosphatæmia produced. They believe that normal serum has low and high threshold values for vitamin D. Above and below these thresholds the values of phosphorus and calcium are respectively excessive and deficient, and therefore excess of vitamin D results in calcification.

An interesting relationship between the toxicity and the antirachitic properties of irradiated ergosterol has been pointed out by Scheunert and Schieblich (1929). Comparing these two properties quantitatively, they found them approximately parallel, indicating that the antirachitic molecule is also the toxic molecule.

In spite of the fact that these results and those of other workers seem to prove an actual toxicity of vitamin D itself when given in excess, Harris and Moore consider that there is little risk to human beings of ill-effects from excessive vitamin intake, since the quantity of vitamin D which proves toxic to a rat, though forming less than 0.1 per cent. of the diet, is yet some 100,000 times an adequate "physiological dose."

This opinion is confirmed by the results of Cartland *et al.* (1929) who conclude that when the vitamin content of the diet is complete and adequate an increase of the vitamin D intake in normal growing rats and dogs, up to a thousand times the minimum effective dose, produces no effect on growth, calcium and phosphorus metabolism,

blood calcium or phosphorus, or on the histologic structure or calcium content of the tissues.

Harris (1930) considers that the occurrence or severity of the hypervitaminosis is determined not only by the degree of vitamin D excess administered and the length of the experimental period, but also by the calcium and/or phosphate content of the diet, and this observation no doubt accounts for a certain amount of confusion which is evident in the literature.

(3) **Demineralisation of the Skeleton.**—Experiments by Brown and Shohl (1930) indicate that vitamin D controls calcification of the skeleton by dissolution and deposition of the bone salts. According to these investigators, doses of vitamin D somewhat larger than the minimum antirachitic amount tend to promote well-being. At any rate, the bone ash in growing animals becomes heavier and the calcium retention greater. When larger, toxic doses are administered, however, pathologic calcification ensues. The symptoms are attended with a lessened concentration of mineral matter in the bones, a shift of calcium and phosphorus from the faeces to the urine, and reduced or negative calcium and phosphorus balances. Rachitic animals are more resistant than normal individuals to toxic amounts of viosterol; and, strangely enough, rickets may be cured without complete return of the affected animals to health. There may be a demineralisation of bones while calcium is being deposited at the epiphyses,—a supposition which is upheld by Watchoru (1930).

It is pointed out by Duguid, Duggan, and Gough (1930) that, since the earliest change in the aorta is a degeneration which may go on to calcification (Huckel and Wenzel, 1929), it is possible that hypercalcaemia is not the essential change. It is not entirely clear whether calcium is the toxic agent or whether some other substance affects the medial muscle, the hypercalcaemia and calcification being merely incidental.

(G) **RECOVERY FROM EXCESSIVE DOSAGE OF IRRADIATED ERGOSTEROL.**—Blunt and Cowan state that if excessive doses of irradiated ergosterol are discontinued, animals usually make a rapid and complete recovery. Appetite is regained, growth and normal weight are resumed, and the abnormal calcium deposits very largely disappear.

XXXIV. STABILITY OF IRRADIATED SUBSTANCES.

Foodstuffs once irradiated are very stable in their antirachitic properties. Daniels and Jordan (1929) have recently carried out experiments which indicate that the antirachitic properties of irradiated foods are not affected by superheating for a period of 6 hours at 15 lb. pressure.

Karóly (1927) found that irradiated wheat flour did not lose its activity after boiling 30 minutes, after baking, or after keeping for at least 2 to 3 months.

Blunt and Cowan (1929) state that irradiated olive oil was unchanged in activity after being kept for months in a stoppered bottle in the dark. Irradiated dried milk kept for 6 months in a cupboard under ordinary conditions had lost but little of its potency, and even after a year it had deteriorated only moderately. Some irradiated spinach and lettuce were just as active after cooking as before. Even the violent chemical procedure of boiling with alcoholic alkali, as in saponification of an irradiated oil, does not destroy the active substance.

The potency is lost when exposure to ultra-violet radiation is carried out too long. Activated olive oil lost its activity after 17 hours' exposure to ultra-violet rays, and so even did cod-liver oil.

Cereals irradiated by the process of Steenbock (1929) showed a surprising stability to cooking and drying which was not in harmony with what is known of the stability of vitamin D in the unsaponifiable fraction of irradiated fats or cod-liver oil.

ANTIRACHITIC ACTIVATION OF CEREALS AND ITS STABILITY TO COOKING.

Cereals.	Average Dry Weight of Femurs.			Average Ash Weight (Femurs).			Average per cent. Ash (Femurs).		
	Un-treated. Mgrms.	Irradiated. Mgrms.	Irradiated and Cooked. Mgrms.	Un-treated. Mgrms.	Irradiated. Mgrms.	Irradiated and Cooked. Mgrms.	Un-treated. Per cent.	Irradiated. Per cent.	Irradiated and Cooked. Per cent.
Wheat	127	128	—	41	59	—	31.7	45.9	—
Patent flour ...	107	127	—	33	61	—	30.7	48.3	—
Cream of wheat ...	112	135	—	31	62	—	27.6	46.1	—
Shredded wheat ...	106	148	—	32	76	—	31.4	55.2	—
Corn meal	128	165	145	29	72	65	22.3	43.6	44.6
Corn flakes	121	135	122	32	58	52	26.7	43.1	42.8
Hominy	110	134	123	31	62	56	27.6	46.0	45.4
Rolled oats	122	158	168	33	73	70	26.8	45.9	41.7

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Further experiments were carried out by Steenbock, Riising, *et al.* (1929) to confirm this stability.

In one series rolled oats, spread out flake by flake under a Cooper-Hewitt lamp, were irradiated, and samples of 300 grms. each were stored in Mason jars at temperatures ranging from 5 to 10 C. up to 45 and 60 C. for more than 2 years. The samples were tested periodically for antirachitic potency by feeding them to rats which had been made rachitic on Steenbock and Black's ration 2965 (1925). In this ration, consisting of 76 per cent. yellow corn, 20 per cent. wheat gluten, 3 per cent. calcium carbonate, and 1 per cent. sodium chloride, 20 per cent. of the yellow corn was displaced by the irradiated cereals. This substitution was made after the rats had been on ration 2965 for about 3½ weeks, by which time they had developed severe rickets as revealed by the enlargement of the wrists and by their peculiar shambling gait. After 10 days on the modified ration, the rats were killed, and the distal ends of the radii and ulnæ were examined for the linear deposition of calcium in their metaphyses. This was done by means of the Johns-Hopkins technic, described by McCollum, Simmonds, Shipley, and Park (1922). A pronounced residual antirachitic potency was found in the rolled oats after 16 months' storage, irrespective of the conditions, hot or cold, light or dark; but after from 23 to 28 months the activity was practically entirely destroyed at the higher temperatures.

PALATABILITY OF IRRADIATED FOODSTUFFS.—Many proteins, fats, and carbohydrates of an ordinary state of purity are changed in taste by exposure to ultra-violet radiation, though the destruction of palatability is stated to be no index of their vitamin D content. The substances responsible for the unpleasant taste are apparently of a stable non-volatile nature, and Steenbock, Riising, *et al.* (1929) state that their formation cannot be prevented by carrying out the process in inert gases *in vacuo*, or at low temperatures, nor can they be removed by aeration.

In the case of *milk*, however, it has already been shown that various modifications of the process have been adopted with some success.

PURE BUTTER FAT, free from protein, does not, according to Röhr and Schultz (1927), acquire an unpleasant taste when irradiated, nor does *salad oil* if the protein is just coagulated by heating to boiling-point.

Cereals have been induced to remain palatable under irradiation by the Steenbock process by reducing the time exposure. Exposures up to 1 minute gave the highest efficiency without producing any change in palatability.

XXXV. OTHER THERAPEUTIC EFFECTS OF VITAMIN D.

(A) **INFANTILE TETANY.**—According to György (1929), tetany is associated with a hypocalcæmia, in contradistinction to the hypophosphatæmia of rickets. Mirglia (1926) has also found that hypocalcæmia is a constant finding in tetany, and that ultra-violet rays are capable of raising the blood calcium level to normal over a long period.

György believes that, chemically, the blood in tetany shows a sort of negative or mirror image of the rachitic manifestations. Thus, in tetany the quotient Ca : P is diminished because of the hypocalcæmia, whereas in rickets it is increased because of the hypophosphatæmia. Similarly, he draws a comparison between the acidosis which he believes to exist in rickets and the alkalosis which, he states, will induce the clinical manifestations in tetany. Clinical studies made by Gleich and Goodman (1928), Rohmer and co-workers (1928), Bakwin (1929), Wilkes (1929), and others indicate that of the group of agents which exert a lasting effect on the clinical manifestations and the serum calcium of infants with tetany, irradiated ergosterol is the most rapid in its effects.

Wilkes states that he has been able to cure it within 8 days, no other treatment than ergosterol having been given.

Brougher (1930) has used viosterol with success in patients suffering from tetany following bilateral lobectomy.

Rohmer and co-workers (1928) believe that the muscular flaccidity and susceptibility to infection are more quickly corrected by irradiated ergosterol than by ultra-violet therapy.

Linden and Harris (1929) report that treatment of chronic tetany, associated with fatty diarrhœa in children with cœliac disease, by an increased supply and better absorption of vitamin D is more promising than the administration of calcium salts.

These results have not, however, been confirmed by Urachia and Popovicini (1928). They concluded from the varying results obtained that irradiated ergosterol has no marked effect in parathyroid tetany, and does not influence calcium and phosphorus metabolism in any constant fashion, nor does it prolong the life of the animals submitted to the operation.

Wilkes and co-workers (1929) found that irradiated ergosterol requires about 3 weeks to cause serum phosphorus and calcium concentration to return to normal, and issue a warning against using irradiated ergosterol in patients with tetany who show a high blood phosphorus content.

(B) **INFANTILE SPASMOPHILIA**, whose connection with rickets has been closely investigated by Stepp and György (1927), has been observed by Gerstenberger and co-workers (1930) to depend upon three factors :

(1) Rickets.

(2) Exposure of the rachitic infant to the influence of the antirachitic factor in one form or another in a sufficient degree to produce some healing.

(3) Interruption of such exposure for an interval usually of from 30 to 60 days, or inadequacy of the exposure, or both. Infantile spasmophilia is a symptom complex, characterised by a hyperexcitability of the nervous system, and rising to a maximum in the spring and autumn. Although practically every spasmophilic infant is rachitic, not all rachitic infants are spasmophilic.

In 1920 Huldshinsky associated the treatment of rickets with the development of spasmophilia, and for a time considered as an explanation the application of inadequate therapy. This theory is revived by Gerstenberger, whose observations show the development of typical spasmophilia in infants (*a*) which had received inadequate treatment in the form of milk from a wet nurse whose skin was exposed daily to the sun for 45 minutes. This milk had anti-

rachitic power, but not in a marked degree, and represented for this rachitic infant an inadequate intake of the antirachitic factor, although it also may have been of an interrupted character; (b) which had received interrupted treatment in the form of either exposure to sunlight or to an erythema-producing exposure of the quartz lamp. The symptoms of spasmophilia developed 60 days after the cessation of treatment. They explain the spring and autumn peaks of spasmophilia on the same hypothesis. "When at the end of winter and in the beginning of spring better weather appears, human beings, including infants, are glad to get out into the open and, as a result, unknowingly are exposed to the action of the antirachitic factor. This exposure may be repeated for a number of consecutive days or weeks and may be sufficient to cure rickets without the development of spasmophilia. Usually, however, it happens that the first periods of good weather are followed by more or less prolonged and irregular periods of cold and cloudy weather which forces a return to indoor life and winter clothes. This change represents, in our opinion, the automatic interruption in the exposure of the skin of the rachitic individual to sunlight and, when it persists long enough, results in the development of the spring spasmophilic picture if the earlier exposure to sunlight has been of sufficient degree to start the healing process."

In the autumn the peak of spasmophilia is less than in the spring, and Gerstenberger explains this by stating that young, growing infants, in whom the chief cases occur in the autumn, have been protected against exposure to the summer sunlight, and as a result have developed mild rickets. Their exposure to the sunlight of late summer and early autumn, and its interruption or withdrawal during the oncoming colder weather, represents the factor of inadequate or interrupted therapy which they consider responsible for the onset of spasmophilia.

Their observations further show that whenever adequate therapy is used in the form of unfiltered quartz lamp rays, the spasmophilia quickly improves, and later disappears completely.

(C) **OSTEOMALACIA.**—The close connection between vitamin D and osteomalacia has been suggested by several workers.

Pommer (1885), in his studies upon the histological changes in osteomalacia and rickets, concluded that the essential abnormality in each disease was a more or less complete cessation of calcification. Such changes as he found to be peculiar to rickets, Pommer attributed to differences in the age of the patients. Schmorl (1905) also found evidence of deficiency of provisional calcification and irregularity of endochondral ossification in young subjects of osteomalacia, changes exactly similar to the disturbances of endochondral ossification in rickets. The general view is that rickets is accompanied by a faulty deposition of mineral matter in young bone that has not yet undergone ossification, whilst in osteomalacia there is a substitution of organic for mineral matter in a bone that is mature. In marked cases of osteomalacia, replacement of the bony tissue of the medullary spaces by osteoid tissue gives rise to a cystic appearance in the X-ray picture.

Hess (1930) believes that the differences in the pathological lesions in rickets and osteomalacia are quantitative rather than qualitative, and that the underlying pathological process is the same. According to Preston Maxwell (1930), true osteomalacia is the manifestation of rickets in bones that have reached maturity, and is connected with a shortage of vitamin D, and in most cases with an actual calcium starvation. It must be distinguished from other diseases causing osteoporosis, such as hyperparathyroidism, pseudo-osteomalacia malignum, and, in rare instances, hyperthyroidism.

It is possible that the so-called physiological osteomalacia of pregnancy (Hanau, 1892) is a mild form of osteomalacia. In most cases the blood calcium is reduced to 5 to 8 mgrms. per 100 c.c. of serum and the blood phosphorus to 1 to 2.5 mgrms. per 100 c.c. The

Ca and P product gradually reaches the normal figure after treatment. The deformity may affect the chest, pelvis, or long bones, and kypho-scoliosis is common.

The chest tends to become "pigeon-breasted," with flattening of the ribs and crumpling of the sternum, reducing the distance from the episternal notch to the ensiform cartilage. In the pelvis the yielding at the acetabulum and at the ilio-pubic and ischio-pubic junctions, together with increased concavity of the sacrum and crumpling of the ischium and pubes, produce a triradiate cavity. In the long bones irregular bending and transverse fractures are common lesions.

In Maxwell's experience, treatment by a daily dose of $1\frac{1}{2}$ to 2 ounces of cod-liver oil, or a milligramme of ergosterol with a teaspoonful of cod-liver oil, gave good results.

An account of the prevalence of osteomalacia in India is given by Kathleen O. Vaughan (1928). Osteomalacia is very common amongst women affected by the Purdah system, which results in their almost complete seclusion from light from the age of 8 to 10 years until after their marriage and the birth of one or two children. The disease is unknown amongst men and boys and women of the lower class who are out all day in the open air.

In China the disease is common amongst women of child-bearing age and can be cured or prevented by improving the diet and giving cod-liver oil. The same treatment was found to be successful in the "hunger osteomalacia" prevalent in Vienna in post-war famine conditions. Cases of late rickets and osteomalacia have been recorded by Wilson and Surie (1930) among men and boys as well as among women in the Punjab. Most of these cases, comparable to the post-war hunger osteomalacia in Central Europe, were found where not only the vitamin D content of the diet, but the calcium and phosphorus and all other constituents were quantitatively deficient also. Wilson and Surie state that excess cereal in the diet plays some part in rendering the condition of the patient worse, and that symptoms do not disappear unless the diet includes fresh food; whether this is due to a previous lack of vitamin C or of the proper acid-base ratio, or of proper salt balance, remains yet to be determined.

Striking improvement in cases of osteomalacia has been reported by Hottinger (1927), Lasch (1928), Green-Armytage (1928), and others following prolonged administration of irradiated ergosterol. Green-Armytage (1928) has found irradiated cholesterol invaluable in the numerous cases of osteomalacia seen by him in Bengal. Pure cholesterol is poured in a thin layer in a petri dish and exposed for one hour to the rays of a quartz mercury-vapour lamp; of this 2 drms. are given twice a day in 4 oz. of liquid paraffin.

Mathez (1927) has also reported a marked amelioration of the symptoms in a case of chronic (12 years) osteomalacia in a man after treatment with a pharmaceutical preparation containing vitamin D. He recommends, by analogy, vitamin D therapy in all cases of epicondylitis with severe pains lasting for years, without röntgenographic or clinical disturbances, and despite treatment.

Infantile Osteomalacia.—The existence of osteomalacia in childhood has been doubted, but of 5 cases of active rickets observed by Blank and Graves (1929), 3, which were post-infantile (aged $3\frac{1}{2}$, 5, and 6 years), showed X-ray changes in the shafts of the bones similar to those seen in adult osteomalacia. All the cases reported responded well to antirachitic treatment.

(D) ARREST OF GROWTH AND MARASMUS.—That rickets is not the invariable result of a diet deficient in vitamin D has been shown by the investigations of Chick and co-workers (1923), who found that in many cases such a dietary deficiency produced only arrest of growth. Essential or primary marasmus, of no known origin, and commencing within two weeks of birth, has been also suggested as a result of vitamin D deficiency. McFadyean (1928) reports a series of cases in which the common cause of secondary marasmus, such as rickets, had been eliminated, and where the additions of vitamins A, B, and C to the diet had failed to produce

any benefit. Administration of vitamin D, in the form of ostelin (the unsaponifiable fraction of cod-liver oil), invariably cured the marasmus and caused the weight curve to follow a normal course. Flamini (1926) has shown the effect of 3 to 5 minims five times daily of the non-saponifiable fraction of cod-liver oil, extracted by Zucker's method, on the body weight of 32 infants. He attributes the increase of weight to the improved intestinal absorption, and to the improved nutrition of the tissues, due to more active assimilation.

Experiments by Goebel (1929) on young rats also illustrate the growth-promoting action of vitamin D. A group of rats on McCollum's 3143 diet gained 31 per cent. of their weight in 3 weeks, while those on a similar diet to which a daily dose of 1/400 mgrm. of irradiated ergosterol was added gained 117 per cent. in the same time. Rats on a normal diet, to which the above dose of ergosterol was added, gained 182 per cent. of their weight in 6 weeks, while those on the normal diet alone gained 117 per cent.

A similar effect of growth was observed by Bacharach (1928) in testing for the presence of vitamin D in soy-bean oil. He found that when ostelin was added to the diet superior growth took place, which he attributed to the action of the vitamin D contained in it.

Observations by Steenbock (1924) and Drummond (1926) on the growth response of animals on a deficient diet to irradiated cholesterol show that it is not in regular proportion to the amount of cholesterol given. In some animals a retardation and eventual cessation of growth may set in after about 4 weeks, and is not restored by a larger amount of irradiated cholesterol. Growth is, on the other hand, resumed when vitamin A is given. These observations suggest that there is a definite relationship between the growth-promoting action of vitamins D and A, and that vitamin D exerts its full growth-promoting influence only in the presence of vitamin A.

(E) **CALCIFICATION OF TEETH.**—The earlier investigations carried out by Mellanby and Pattison (1918–1928) on the influence of vitamin D on dental decay were not universally accepted as conclusive evidence. In their earlier work they found that food substances which contained the antirachitic or calcifying vitamin, now called vitamin D, greatly stimulated the calcification of teeth, while cereals, and especially oatmeal, inhibited perfect calcification of the teeth when this vitamin was deficient in the diet. Similarly in children they showed that diets favourable to calcification limited the initiation and spread of caries, while diets of lower vitamin content, and containing oatmeal, had no such effect. In many cases also diets rich in vitamin D caused "hardening" of teeth in which caries had started, and thus tended to suppress the active carious process. On the other hand, diets with low calcifying properties had no such retarding influence.

In later experiments an attempt was made to vary only the vitamin D factor in the food. The experiments were made on 21 children, who were given a diet with plenty of milk, butter, meat, and fruit. Each child received in addition a preparation of irradiated ergosterol in amounts varying from 2 c.c. to 4 c.c. The mouths were carefully charted before the commencement of the experiment, and the number of carious teeth noted, and also the condition of the cavities. The feeding was carried out for 28 weeks, and the dental conditions again noted. Comparing the results with those obtained in 1926, in children who were divided into three groups and given a constant diet, with variables in the shape of fat-soluble vitamins and the anticalcifying substances contained in cereals, it was found that the initiation of fresh cavities was less in the children given ergosterol only, as compared with the earlier investigation. Even those children in that experiment who received extra fat-soluble vitamins had more fresh caries than the last group who received pure vitamin D. Similarly, the figures for the spread of existing cavities and the "hardening" of caries were more favourable in the children

given ergosterol than in the 1926 test. On the basis of these and other experiments, Mellanby and Pattison suggested that vitamin A plays little if any part in lessening the liability to caries, and that any such action is essentially due to vitamin D.

The above results were criticised by various upholders of the theory of the production of caries by a fermentable carbohydrate diet. Sim Wallace (1928) pointed out that the groups of children used as controls received more sugar than the group receiving radiostol, and that associated with variations in the amount of sugar and fermentable carbohydrates there went a corresponding variation in the amount of caries.

From Malay and South Africa also have come statements which cast doubt on the "avitaminosis" theory of dental caries. J. W. Field (1929) states that though the Chinese live on a diet which should ensure a sufficiency of vitamin A and D, and though they are exposed to powerful solar irradiation, dental caries is prevalent amongst them. The diet of the Tamils, on the other hand, is a poor source of vitamins A and D, while they suffer rarely from caries. Field suggests that the difference lies in habits of dental hygiene. The Chinese rarely clean their teeth, so that soft carbohydrate food collects in the pits and crevices, while the Tamils clean their teeth with charcoal and also chew the fibrous areca nut.

W. Fox (1929) also states that dental caries is extremely common in South Africa, both among the native and European population, where sunshine is specially abundant and rickets is almost unknown.

Agnes H. Grant (1926) has carried out some investigations which seem to show that the normal development of teeth requires not only a sufficient amount of vitamin D, but also an adequate balance between vitamin C and vitamin D and the amount of calcium in the diet.

When there was an adequate balance between vitamins C and D in the diet both enamel and dentine were normal. Deficiencies of both vitamin C and D affected both dentine and enamel; and the abnormalities were exaggerated by an excess of Ca (as calcium lactate). When vitamin D was given without vitamin C, an excess of Ca caused the teeth to grow so abnormally hard that they would not wear down. When vitamin C was given without vitamin D, Mg was deposited in the place of Ca to such an extent that the incisors became very brittle and broke off frequently. An excess of Ca exaggerated this condition.

The suggestion has been put forward from several sources that rickets predisposes to dental decay. The Medical Officer of Birmingham, E. H. Wilkins (1927), reports that children with severe rachitic deformities have a greater proportion of carious teeth than those with milder degrees.

The Report of the American Public Health Association Committee emphasises the importance of giving cod-liver oil to infants during the first two or three years of life, together with exposure to the rays of the sun, for the eradication of both rickets and dental caries.

Hess and co-workers (1929) consider that irradiated ergosterol may improve the calcification of the teeth if given during the stage of their development; in respect to the deciduous teeth this period would have to embrace prenatal life. In 1929 Weinmann, of Vienna, published a short account of an investigation that is of interest in this connection. He showed that in rats it is possible by means of very large doses of irradiated ergosterol to induce a hypercalcification of fully developed teeth.

Whether vitamin D is the sole factor in the prevention of caries or whether other factors, both dietary and inherent, are equally concerned, cannot at the moment be definitely stated, but taking all aspects of the question into consideration the possibility should not be over-

looked that a deficiency of calcium may exist alongside a deficiency of vitamin D in diets which favour dental caries.

Mellanby's summary (1930) of the relation between vitamin D and calcium and phosphorus intake is as follows: "(1) When the vitamin D intake is high and the calcium and phosphorus intake low, perfect calcification of bones and teeth will ensue. (2) When the vitamin D intake is moderate, increasing the calcium and phosphorus intake will improve the calcification of bones and teeth. (3) When the vitamin D intake is very low, however high the calcium and phosphorus intake, imperfect calcification of bones and teeth will result, although not as imperfect as would have been the case had the calcium intake been low." He emphasises also the toxic factor in cereals, particularly oatmeal, which interferes strongly with the deposition of calcium and phosphorus in teeth. He points out that the relation between caries and the structure of the teeth depends not only on the original formation of the teeth but upon their resistance to bacterial onslaught in later life, which again depends upon the diet (*i.e.* one with deficient vitamin D and much cereal). Thus a child may have a poor diet resulting in badly formed teeth, but at a later stage the calcifying qualities may improve and so increase the resistance of the teeth to caries. Similarly, well-formed teeth may lose their resistance when the diet becomes defective and so become more susceptible to caries.

(F) **ANTIBACTERIAL RESISTANCE.**—The lessened resistance towards infection by *B. suispestifer* in rats with experimental rickets, can, according to Maurer and Hofmann (1928), be raised three to fourfold by the addition to the diet of 3 per cent. of irradiated sesame oil or 3 per cent. cod-liver oil.

Robertson (1929), however, states that cod-liver oil has no effect on raising the resistance to *B. coli* infection though irradiated ergosterol probably does, as also does exposure to sunshine. Robertson concludes that the increased resistance probably depends upon the curing of rickets. The importance of vitamin D in increasing the resistance of adult as well as growing animals is shown by a recent experiment by Eicholz and Kreitmar (1928), which emphasises also the fact that the influence of vitamin D in this respect is non-specific.

Of 48 full-grown rats on the Pappenheimer-McCann-Zucker diet, 24 were given *per os* daily doses of 0.002 mgrm. of irradiated ergosterol (vigantol, Merck). After 1 month all but 2 were alive and well; of the untreated all but 4 had succumbed to cannibalism and paratyphoid. Two series of full-grown mice on the same diet and others on a normal diet were inoculated with a virulent pneumococcus. The control animals along with those receiving vigantol had a lower mortality than those on the rickets-producing diet. Animals receiving some brewers' yeast instead of vigantol were equally resistant, and those receiving both supplements survived in largest number.

(G) **TUBERCULOSIS.**—Agnes Grant and co-workers (1925) claim to have induced tuberculosis in the otherwise very resistant albino rats by feeding them on a rachitogenic diet. They found that rats from normal mothers receiving an abundance of calcium and cod-liver oil in their diet were immune to as much as 2 mgrms. of bovine tubercle bacilli injected subcutaneously. By using a diet (high cereal type) deficient in both calcium and vitamin D, unmistakable tuberculous lesions were produced in the lungs of rats injected with 0.2 and 0.5 mgrm. of tubercle bacilli. Most of the injected rats developed marked rickets, though not of such a severe type (with scoliosis) as was present in the control rats on the same diet. Most of the inoculated rats grew faster and lived longer than the controls, and it appeared that tuberculosis stimulated this growth. Rats were inoculated within 3 to 7 days after weaning, and the external lesion reached its maximum extent at 5 to 6 weeks and then gradually subsided. No tuberculous lesions were found in any other organ but the lung.

In another series of experiments by Grant, Bowen, and Stegeman (1927), the deficiency of vitamin D was prolonged by beginning the experimental diet with the mothers. The diet was

similar to that of the first series with the addition of bonemeal which provided adequate calcium. The first and second litters raised under these conditions were normal in development, third litters developed slight signs of rickets, whilst fourth and fifth litters developed marked and severe rickets. The reduction of vitamin D in the diet decreased the resistance of the rats to tuberculosis. The amount of tuberculosis which developed seemed to depend upon the inherited vitamin reserves, or upon the susceptibility when weaned, more than upon the severity of the rickets which set in after weaning. Many of the positive cases of tuberculosis were found in the larger rats which had the lightest rickets. The rapidity of growth in the tuberculous rats appeared to retard the development of rickets. The production of rickets was hastened by a cold, gloomy winter, until first litter rats from normal mothers developed rachitic lesions. At the same time the resistance to tuberculosis decreased, so that, by the spring, rats were so susceptible to a vitamin D deficiency that the complication of tuberculosis increased the severity of the rickets. The amount of tuberculosis which developed in young rats followed the susceptibility to rickets very closely. A few cases of tuberculosis appeared in rats in spite of there being cod-liver oil in the diet of the mother, which suggests that there are other factors concerned in increasing bodily resistance to tuberculosis. The tuberculosis of the lungs developing in this series of rats was chiefly of a chronic type and of a form most nearly resembling human tuberculosis.

Platonov states that the subcutaneous injection of *oleum jecoris* and its preparations produces a vitamin-like effect in tuberculosis, the action of sodium morrhuate being greater than that of *oleum jecoris*.

A recent investigation of the rôle of vitamin D in the treatment of tuberculosis has been summarised by Kramer, Grayzel, and Shear (1929). Having in mind the favourable result of ultra-violet irradiation on intestinal tuberculosis, of heliotherapy in other forms of the disease, and the reputed value of cod-liver oil in tuberculosis, they attempted to determine which of the vitamins A and D, in which cod-liver oil is so rich, is responsible for the beneficial effect in tuberculosis. Fifty-eight patients were studied, and cod-liver oil, irradiated cholesterol, cod-liver oil concentrate, and irradiated yeast, together with orange or tomato juice, were compared in the effect on intestinal tuberculosis. The results indicate that all of the substances, with the exception of irradiated cholesterol, are at least as effective as artificial heliotherapy. This outcome suggests that perhaps some constituent of the cod-liver oil other than the antirachitic factor is the effective agent. In order to test the efficacy of vitamin D, apart from the considerable amount of fat and vitamin A of the oil, and apart from the vitamins in the orange and tomato juice, two groups of tuberculous children were compared. Both received a well-balanced diet, but to one group was given, in addition, viosterol. After 12 months of this treatment, with careful clinical supervision, the investigators conclude that the large doses of viosterol employed by them "did not produce any detectable acceleration of the healing process." These observations suggest that such therapeutic value as cod-liver oil possesses in tuberculosis does not depend on its relatively high concentration of vitamin D.

Further studies made by Grant (1930) on the value of cod-liver oil in the treatment of tuberculosis confirm the results previously reported; that, while the addition of vitamin D to an adequate diet increases the resistance to tuberculosis, the addition of vitamin D to a diet that has been markedly deficient in this factor rapidly lowers the resistance. The result of these studies justifies the tentative conclusion that some other factors than an abundance of calcium and vitamin D in the diet are needed to raise an already low resistance to tuberculosis. Resistance to tuberculosis can be decreased from a natural immunity to moderate susceptibility, without destroying the growth impulse, by a prolonged disturbance in the optimal balance that should exist between the calcium, vitamin C, and vitamin D

of the diet. A prolonged excess of vitamin D in the diet, either with a deficiency of calcium or with a normal amount, hastens the spread of tuberculosis through the lungs. The substitution of vitamin C for vitamin D at the time of inoculation, or the addition of vitamin C to a diet which is deficient in vitamin D, tends to increase the amount of tuberculosis in the lungs rather than to decrease it. The addition of vitamin C to a diet containing vitamin D tends to correct the diet and to increase the resistance to tuberculosis. The degree to which diets corrected after inoculation can retard the spread of tuberculosis in rats depends on the type of deficiencies and excesses in the diet before inoculation, and on how long the deficient diets have been given.

(1) **Calcification of Tubercular Lesions.**—Following the numerous demonstrations of the calcifying action of large doses of irradiated ergosterol, several workers have suggested that administration of vitamin D may be beneficial in some forms of tuberculosis, owing possibly to the action of vitamin D in fixing calcium. In comparing the estimations of blood calcium and urinary calcium excretion in groups of patients treated with (1) intravenous injections of Ca and ultra-violet irradiation, (2) intravenous Ca and irradiated ergosterol by mouth, (3) intravenous Ca alone, Caruzzi (1929) found the following responses: (1) Small increase in serum Ca; small decrease in urinary Ca. (2) Serum Ca increased by a few milligrammes per cent.; remarkable decrease in urinary Ca excretion. (3) No significant change. He concludes that in states of demineralisation, and therefore in tuberculous and pre-tuberculous diseases, calcium treatment should be augmented by ultra-violet irradiation, or, better still, by giving irradiated ergosterol.

Levaditi, Li Yuan Po (1930), and Spies (1930) have carried out experiments on rabbits infected with tubercle bacilli (human in Levaditi and Po's investigation, bovine in that of Spies). The results of vitamin D therapy were similar in both cases. Calcium deposits were found in the necrosed and caseous tubercular foci, resulting from the confluence of intracellular calcium concretions. Levaditi and Po suggest that the formation of such a calcium shell may impede the spread of infection to surrounding tissues.

Similar accumulation of fixed mineral matter, composed mostly of calcium salts, has been found by Policard and co-workers (1930) in the tubercular lesions of guinea-pigs.

Calcification of the lungs in both tubercular and normal animals is stated by Simonnet and Tanret (1930) to occur following the administration of ergosterol.

(2) **Advanced Tuberculosis.**—The value of vitamin D in advanced tuberculosis has been emphasised by Menschel (1930). He states that treatment with viosterol brings about the formation of connective tissue and induration, reduces the fever and night sweats, prevents pulmonary hæmorrhage, and promotes healing of tubercular lesions in bone, larynx, or skin. No effect on the accompanying secondary anæmia was observed.

(H) **RESISTANCE TO PARASITISM.**—It is stated by Ackert and Spindler (1929) that the lack of vitamin D lowers the resistance of chickens to the intestinal nematode, *Ascaridia lineata*. In one experiment, 80 per cent. of the chickens receiving vitamin D (by means of daily irradiation with a Cooper-Hewitt lamp and aerated cod-liver oil) freed themselves of their parasites, while only 30 per cent. of the group lacking vitamin D did so. The remaining experiments, however, gave no conclusive evidence that the elimination was due to the influence of vitamin D.

(I) **ACIDOSIS.**—Since the acidity of the blood is diminished by administration of vitamin D, Flamini (1926) advises its use in the treatment of symptoms due to acidosis, such as cyclical vomiting, infantile eczema, and spasmophilia. In the latter condition, Flamini attributes the cure to the increased penetration of calcium into the cells of the sympathetic.

(J) **PROGRESSIVE MUSCULAR ATROPHY.**—Striking improvement in 2 cases of severe progressive muscular atrophy have been recorded from Calcutta by Thomas (1928) from the

administration of parathyroid extract, calcium, and vitamin D, in the form of ostelin and cod-liver oil.

(K) **DEMENTIA PRÆCOX.**—The hæmoclastic crisis which has been shown to occur in 94 per cent. of cases of dementia præcox has been stated by Rees Thomas (1928) to be definitely affected by administration of vitamin D in the form of radiostol. The dose required to establish a permanent reversal of the crisis (which occurs also in hepatic disorder and certain chronic gastric conditions) is stated to be 2 mgrms. (1/33 gram). There was no evidence in the control case of any deficiency of vitamin D obtained from the skin and from the food supplied. Ultra-violet radiation did not produce a complete reversal of the crisis, but required to be supplemented with at least 1 mgrm. of irradiated ergosterol daily. Rees Thomas suggests that the association between vitamin D and mental disorder may lie in some as yet unknown function of the liver, possibly some loss of its ability to store ergosterol.

(L) **DEVELOPMENT OF THE EMBRYO.**—Both in animals and human beings evidence is accumulating to show that a relation exists between vitamin D and embryonic development.

Hess, Bills, and co-workers (1928), investigating the production of hens' eggs, have found that the concentration of vitamin D varies according to the diet and exposure to ultra-violet rays. Groups of hens fed on a vitamin D-deficient diet laid comparatively few eggs, and these contained little antirachitic factor. Fertile eggs from these hens failed to hatch, and the hens' livers were also low in antirachitic potency. Experiments on young chicks tended to show that the lack of hatchability was a phenomenon due to lack of development of the embryo when the hens' diet was deficient in vitamin D, and was to be attributed to defective absorption of calcium from the shell into the yolk. Schultz (1927) has also shown that irradiated milk-fat preparations increased the weight and hatchability of hens' eggs.

Further studies on fish by Hess and co-workers (1929) showed that the roe contained as much, or even more, of the antirachitic factor than the liver, while the newly hatched fish were practically devoid of it. They suggest that the antirachitic factor had been used up in the course of development of the larvæ, and that the results of these experiments may point to vitamin D consisting of two separate factors.

Compared with the number of animal experiments carried out, the number of investigations into the effect of vitamin D on the development of the human embryo is small.

Abels (1927) quotes a case where a course of cod-liver oil during the second pregnancy resulted in the second child weighing 60 per cent. more than the first.

Similar results were obtained by Poullson where a woman, who had previously had seven children (four of which had died at birth), was given a course of cod-liver oil during pregnancy with the result that the eighth and ninth children were born alive, weighed 3250 and 4500 grms. respectively, and developed normally.

Vogt (1928) reports that normal pregnancy and the satisfactory development of the offspring follows the administration of vigantol and levurinosé (which contains vitamins B and D).

(M) **DRESSING FOR WOUNDS.**—The clinical application of a solution of ergosterol in liquid paraffin to a lacerated wound of the upper arm has been described by Bond (1928). He obtained good results also with varicose ulcers treated by this method. Bond observed that irradiation of ergosterol produced a fat-soluble substance containing oxygen, some portion of which could be readily liberated and carried through the lipid material of the cell and its nucleus. He also found that ozonisation of an ergosterol film converted ergosterol into an oxidising substance, probably a peroxide or ozonide of ergosterol. Whether this substance is identical with vitamin D, or whether the formation of the peroxide is a subsidiary reaction

during the process of vitamin D production is not quite clear, but Bond suggests that the beneficial action of irradiated ergosterol on wounds and granulating surfaces may be due to the peroxide rather than to vitamin D.

(N) **GYNECOLOGICAL DISORDERS.**—The good results obtained with irradiated ointments in ulcerative and inflammatory processes of the skin suggested to Polzl (1930) that irradiated fats in the form of suppositories might be used for various forms of vaginitis. He obtained good results in young women with no other evident complications. After the treatment had been continued for about 3 weeks, the discharge and the burning pain entirely disappeared. Less favourable were the results in cases of chronic gonorrhea and in disorders of the uterus, or of the adnexa, though prolonged treatment produced some improvement. Polzl states that menopausal pruritus was also favourably affected.

(O) **OZÆNA.**—Certain cases of ozæna, associated with a general debility, have been stated by Birkholz (1928) to be benefited by the administration of irradiated ergosterol.

(P) **CONSOLIDATION OF FRACTURES AND DECALCIFICATIONS.**—Irradiated ergosterol has been stated to have a good effect in the delayed or imperfect union of fractures in old people and children, and in all conditions, such as pretuberculosis, convalescence from severe illness, and certain chronic rheumatic affections at the menopause, which are accompanied by decalcification of the epiphyses. These effects have been confirmed by Bors (1928), Ferrero (1918), Roi (1930), and Collazo *et al.* (1930) in animals, and by Knoflach and Bond (1928) from clinical observation. Hellner (1928) and Lopez (1930), however, found no difference between animals treated with viosterol and the controls, and Lewis (1930) found no hastening of union or callus formation in 17 cases of fractures in adults.

Morelle (1929) has pointed out that with the exception of Ferrero, all those observers who have found increased consolidation have used a dosage of ergosterol far in excess of the therapeutic dose. He employed, therefore, a dosage of approximately 3 or 4 times the therapeutic human dose (0.1 mgrm.). The radiographic appearance of the callus of peroneal fractures in rats treated with these small doses was found to correspond on the 20th day to that of controls at the 30th day. He concluded that while irradiated ergosterol in large doses produced a denser and more exuberant callus than normal, small doses had the effect of hastening the repair of fractures.

Roederer (1929) also reports rapid consolidation of fractures in patients treated with irradiated oil; 7 days for a fractured clavicle, and 3 weeks for a greenstick fracture of the diaphysis of the humerus. He states that the callus was rapidly absorbed.

Marfan and Dollfus Odier (1929) consider that one of the most important effects of irradiated ergosterol is that of promoting normal calcification in bone.

Comparing its administration with that of ultra-violet light rays, they state that while ergosterol appears to calcify more rapidly, the ultra-violet rays modify more rapidly and more completely the form of the bone. The general eutrophic action of both is very evident, but ergosterol has the advantage of being in a form easily employed and less expensive.

(Q) **STIMULATION OF THE SYMPATHETIC NERVOUS SYSTEM.**—Brunetti (1928) has put forward a hypothesis that vitamin D has a "tonic" action on the vegetative nervous system by virtue of its power of fixation of calcium. He tested the effect of administration of an extract of cod-liver oil for 8 to 20 days on the local reaction following intracutaneous injections of adrenalin and pilocarpin. He found an accentuation of the reaction of adrenalin and a diminution of the reaction to pilocarpin. In the case of adrenalin, he suggested that the deposition of calcium in the sympathetic nervous system brought about by the action of vitamin D renders it more sensitive to adrenalin. In the case of pilocarpin, he explains the diminished reaction (less marked than the increase in the adrenalin reaction) by the

supposition that the tonus of the vagus is also slightly increased by the action of vitamin D, and that therefore the co-existing stimulation of the sympathetic is not sufficient to inhibit the vaso-dilatory action of the pilocarpine.

Flamini (1920) also states that vitamin D raises the blood pressure.

Corlette (1928, 1929) has postulated that many disturbances of nerve function may be due to lack of calcium and that vitamin D may be as truly antineuritic as vitamin B. He suggests that vitamin D deficiency may have a special action on the autonomic nervous system, and gives as an instance the "pot-belly" of rickets arising from abdominal distension due to lesions of the visceral nerves. He states that the liability of stall-fed horses to impaction of the stomach and tympanic distension and the predisposition of the horse to colic probably arise from lack of calcium or vitamin D, and that the disturbance of nerve function produced by calcium deficiency is very wide, and, like lead poisoning, may affect different nerves in different individuals and species.

(R) **PERNICIOUS ANÆMIA.**—Rosenow (1927) reports that irradiated ergosterol appears to improve those cases of pernicious anæmia in which a diet containing liver has not proved beneficial.

(S) **RADIATION SICKNESS.**—The clinical results of the oral administration of viosterol in early radiation sickness are described by Smith (1929). Marked relief of all symptoms is usually effected, except possibly diarrhoea. Used as a prophylactic, viosterol prevents all symptoms to a marked degree. These results apparently occur in both radium and X-ray treatment. Many of the patients experience an unusual sense of well-being and increased appetite. Smith suggests that the action is probably effected in some way through the calcium-phosphorus metabolism. There is some evidence that the final effect may be brought about through prevention or reduction of hyperirritability of the vagus.

(T) **RETARDED BLOOD COAGULATION.**—It is stated by Brougher (1930) that when the blood coagulation time is unduly long, as in acute infectious syphilis, obstructive jaundice, and diseases of the hæmatopoietic system, it can be brought to normal within 4 hours by cod-liver oil or viosterol given orally.

XXXVI. VITAMIN D IN CERTAIN FOODSTUFFS.

(A) **COD-LIVER OIL.**—Not only does cod-liver oil show great variability in its content of vitamin D as compared with vitamin A, but different samples exhibit great difference in antirachitic potency.

Lesné, Clément, and Simon (1928) have reported experiments in which four different samples of Norwegian cod-liver oil, fed at the rate of 2 drops daily to rats on a rachitogenic diet, failed to secure freedom from rickets, though the same dose produced good growth and freedom from xerophthalmia in rats on a vitamin A-free diet. No samples of oil were found which were rich in vitamin D and poor in vitamin A. The fact that the content of vitamin A in cod-liver oil bears no relationship to that of vitamin D is explained by the fact that A is less stable than D and more likely to be affected by heat and oxidation.

(1) **Vitamin D Potency Tests of Various Samples of Cod-Liver Oil.**—Various methods have been used in testing the antirachitic potency of different samples of cod-liver oil.

(a) Leigh Clare and Soames (1928) take as their criterion (1) the increase in bone calcification after small doses in young rats on special diets; (2) the determination of the lowered ash content in the bones of animals on a special diet "F" described; (3) the increase in body weight.

(b) Heuser and Norris (1926) have measured the potency by growth, occurrence of rickets and bone analysis. Oils of American origin, whether refined or unrefined, gave results superior to those of Norwegian origin.

(c) Jephcott (1928) urges the greater accuracy of the determination of the faecal pH, and has found a very great variation in oils tested by this method.

(d) The method suggested by Bills (1925) takes the line test as its criterion. McCollum's 3143 diet is fed to rats for 18 or a few days more after weaning, and then the test preparation is given for exactly 5 days, taking care to use only rats that eat satisfactorily and do not lose weight. The "minimum curative dose" is the amount of the oil required to produce definite but not extensive healing. He distinguishes 4 degrees of healing by one to four plus signs. As a standard he takes the average activity of Newfoundland medicinal cod-liver oil. On the average this produces distinct (2 +) healing at a level of 0.25 per cent. of the diet. He calls this standard 100 and compares other oils on this scale.

(e) The Council on Pharmacy and Chemistry of the American Medical Association have adopted the standard of one "rat unit of vitamin D" as "that amount of vitamin D which, when uniformly distributed into the standard vitamin D-deficient diet—ration 2965, (see p. 182)—will produce a narrow and continuous line of calcium deposits on the metaphyses of the distal end of the radii and ulnæ of standard rachitic rats." A "potent cod-liver oil" contains one of these rat units in every 0.75 mgrm.

(f) Coward's (1928) standard preparation of irradiated ergosterol, in terms of 0.0001 mgrm. unit, may be used as a measurement. A good oil may contain 150 units per gram and the poorest 50 units with intermediate values of 70 to 100 units respectively.

(2) **Factors affecting the Potency of Cod-Liver Oil**—(a) **CONDITION OF THE LIVER.**—Hess, Bills, and Honeywell (1929) have carried out a detailed investigation into the ratio between the antirachitic potency of the oil and the size of the liver of the cod. They have concluded that, for a fish of given size, the antirachitic potency varies immensely with the amount of oil in the liver. Although the oils that proved high in potency were obtained from small livers, these livers were large in comparison with the very small amount of oil that could be extracted from them. The most potent oil, assaying at 20,000, was extracted from a liver that weighed 45 grms., contained only 0.27 gram of oil, and had a liver-oil ratio of 167. No relationship could be established between sex and potency. Oils from the livers of individual cod may vary 1000 times in their antirachitic value, and the most potent oils, which may be 200 times as potent as the oil heretofore considered "high grade," are very dark, and can be obtained only in small amount by extracting the liver with solvents.

One of the most interesting questions that these observations raise is how the vitamin or antirachitic factor so readily soluble in fat remains intact in the liver while the fat of this organ is consumed. Hess and co-workers believe that this phenomenon results from an oxidation of the liver fat, which comes about in the course of starvation. The fat is used up in the liver of the cod very much as Miescher (1897) and Greene (1926) found it to be consumed in the muscle of the salmon during the spawning period, when the fish take practically no food. In the cod the antirachitic factor resists oxidation, remains behind, and becomes concentrated in a liver that is shrunken and greatly impoverished.

(b) **IRRADIATION.**—Irradiation of cod-liver oil has apparently no effect on its antirachitic potency. Wyman and co-workers (1926) have shown that a medicinal cod-liver oil radiated $\frac{1}{2}$, 1, and 2 hours possessed antirachitic properties no greater than the original oil, as determined by blood Ca and P, by radiographs, by growth curves, and by post-mortem examinations.

Daniels and Brooks (1927) and van Leersum (1930) have come to the same conclusion, the latter stating that the original activity is even somewhat decreased by irradiation.

(B) **COD-LIVER MEAL.**—Cod-liver meal obtained from the dried livers after extraction of oil varies in antirachitic potency, but, according to Bethke and co-workers (1928), Cruickshank and others (1927), it cannot be regarded as a good substitute for cod-liver oil. Stuart (1928)

states, however, that cod-liver meal and cod-liver oil are quite comparable in promoting growth.

(C) **OTHER FISH OILS.**—Vitamin D occurs in the oils of many species of fish, the quantity varying widely even in those closely related.

Experiments by Bills (1927) have demonstrated that it is not increased by irradiating the fish, nor decreased by keeping them on a vitamin deficient diet.

It has been suggested by Williams and McLennan (1929) that margarines should be enriched in vitamin D content by the addition of fish liver oil.

Not only the liver but the body oils of certain fish are active.

(1) **Puffer fish oil**, prepared by steam distillation of the puffer fish (*Spherooides maculatus*), has been shown by Hess and Weinstock (1926) to have an antirachitic effect at least 15 times as great as the most active specimens of cod-liver oil. A batch of the live fish were kept in a tank for 3 months in a closed building from which all ultra-violet radiations were excluded; their food consisted largely of herring. At the beginning of the test period less than 5 per cent. of oil could be extracted from their livers, whereas after the 3 months 55 per cent. of oil could be extracted: the antirachitic potency of the oil was as great at the end of the test as at the beginning when the fish were caught in the open sea. It is inferred that the antirachitic factor can be "elaborated in undiminished potency" by fish deprived of sunlight for long periods and that this characteristic quality of fish liver oil is dependent on the diet of the fish.

(2) **Burbot fish oil** has been tested by Clow and Marlatt (1929) and found to be about 8 times as potent as cod-liver oil, though, in view of the investigations of Hess and co-workers (1929) into the antirachitic ratio of cod-liver oil, there is doubt if this is due to inherent proportions of the oil or to variations in the individual samples.

(3) **Herrings and Sardines.**—According to Bills (1927), four samples of herring from Newfoundland and four samples of sardines from California gave oils with a potency of 100. Herring meal was found by Baerøe (1926) to be a good preventive against rickets in young swine, 150 grms. being equivalent to 15 grms. of cod-liver oil. Kipperred herrings are also stated by Schmidt-Nielsen (1929) to be a good source of vitamin D.

As the fat content of herring averages 7.1 per cent. of the edible portion, and one might readily eat the flesh of from $\frac{1}{2}$ lb. to 1 lb. of the fish, the 9 to 18 grms. of oil furnished will give a good dose of vitamin D. Sardines have about the same percentage of fat and so they also can compete with cod-liver oil.

(4) **Salmon.**—Salmon body fat is potent— $\frac{1}{8}$ th as potent as cod-liver oil. Salmon oil, extracted from cannery refuse, a considerable proportion of which is the viscera of the fish, was found by Davis and Beach (1928) to protect chickens from rickets in a dosage of 2 per cent. of the total diet.

(5) **Seal.**—A sample of the blubber oil of an adult seal is only slightly antirachitic (3 on Bills' scale), and that of the newborn seal not at all.

(6) **Shell Fish**—(a) **OYSTERS.**—Experiments by Jones, Murphy, and Nelson (1928) show that oysters are a good source of vitamin D. Five grams. of oysters given to rachitic rats daily for 10 days induced slight calcification of the long bones, comparable in degree with that produced in the same length of time by 4 mgrms. of good cod-liver oil. The same daily quantity of oysters induced about half calcification in 15 days and complete calcification of the rachitic metaphyses in 20 days.

(b) **CLAMS**, both the soft and the hard shell variety, are also stated by the above workers (1928) to contain vitamin D.

(D) **ANIMAL FATS**—(1) **Butter.**—The difference in antirachitic potency between butter and cod-liver oil is much greater than that of their vitamin A content. Jones, Steenbock,

and Nelson (1924) have determined a relative value of approximately 1 to 200 ; these values will vary, however, with the seasonal conditions under which the butter fat and the cod-liver oil are produced, and the method of preparation of the latter.

Other samples of spring and summer butter assayed by Coward (1927) contained only from 0.8 to 1 unit of vitamin D per gram, as compared with 50 to 150 units for cod-liver oil. Flury (1928) also confirms the low antirachitic potency of butter.

(2) **Fat of Marine Mammals.**—Experiments by Matzko (1929) indicate that the subcutaneous fat of the seal and dolphin contains significant amounts of vitamin D, both the curative and prophylactic effects of experimental rickets obtaining with a dose of 0.15 gram per day, which is equivalent in the case of the seal to 1.4 to 2.1 per cent., and in the case of the dolphin to 1.5 to 2.9 per cent. of the food consumed. This compares favourably with the results reported by McCollum and co-workers for good cod-liver oil.

(3) **Pulmonary Fats.**—Lung fat has been shown by Roger, Binet, and Vagliano (1924) to contain vitamin D as well as vitamin A. Healing of rickets in young rats was produced by the replacement of butter in a rickets-producing diet by 2 per cent. of fats extracted from the lungs of the dog by means of alcohol and ether.

(E) **MILK.**—The antirachitic potency of milk in general is not high. Quantitative estimations by the Pharmacological Laboratory of the Pharmaceutical Society, under the direction of Dr. J. H. Burn (1930), have yielded the interesting result that the best samples of milk have only a five-hundredth the vitamin D content of a good average sample of cod-liver oil. The antirachitic action of 20 drops of cod-liver oil is therefore equivalent to that of at least a pint of good milk.

According to Honeywell and co-workers (1930), 12 c.c. of raw milk daily are required to prevent the decrease in ash content of the femur during a 21-day feeding period.

As in the case of vitamin A, the vitamin D in milk has been shown by Crawford, Golding, Perry, and Zilva (1930) to be associated with the fat and to be no more concentrated in one fraction of the fat than in another.

According to most observers, the antirachitic potency of milk of one species does not hold for another. Hess and Weinstock (1924) have shown that human milk cannot protect rats from rickets, while cows' milk can. Their observations have been confirmed by Outhouse and co-workers (1928), who have worked out a quantitative comparison of the antirachitic effect of cows' and human milk upon rats. It was found that human milk fed at the rate of 25, 30, or 40 c.c. per day showed no evidence of any antirachitic factor, whereas 30 c.c. per day of cows' milk induced marked healing of rachitic lesions in rats in 7 days.

On the other hand, although rickets sometimes develops in infants on the normal diet of human milk, and when living under normal conditions, human milk is definitely superior to cows' milk in preventing infantile rickets.

Specially good results have been reported from the use of Weissenberg's Citric Acid Whole Milk, which is stated by Behrens (1929) to be very easily digested, simple, and inexpensive prophylactic against rickets.

On the basis of these facts, Hess and Weinstock (1927) suggest that the high protective power of human milk in human rickets should be ascribed not so much to its content of vitamin D as to its superior content of lactose (6 to 7 per cent.), which may tend to promote acidity of the intestinal tract with a consequent increase of Ca absorption.

More recent experiments by Sherman and Stiebeling (1929) emphasise the importance of the liberal supply of mineral elements contained in milk. In many vitamin studies the diets are often inadequate in mineral as well as vitamin content. Sherman and Stiebeling have used a "synthetic" diet comparable to that formulated by Osborne, Mendel, and Park (1923, 1926), which can be altered to make the diet vary in its potential acidity and alkalinity. They

report "that when the young reared in families whose dietaries include milk to the extent of from 25 to 40 per cent. of the calories are transferred at 21 or 28 days of age, soon after weaning, to a basal diet otherwise adequate but practically free from vitamin D, from 5 to 10 per cent. of their calories coming from whole (summer) milk powder carried sufficient vitamin D to foster normal growth and calcification. Still smaller allowances of milk resulted in corresponding effects on calcification."

Factors in increasing the Antirachitic Potency in Milk—(a) MATERNAL DIET.—The question of increasing the antirachitic value of the milk through the diet of the mother is one upon which there is not quite definite agreement.

The chief extraneous substances whose inclusion in the diet has been claimed to increase the antirachitic potency are :

(i) *Cod-liver Oil*.—Hess and Weinstock (1927) have stated that cod-liver oil cannot be relied upon to protect the human offspring from rickets, but McCollum and co-workers (1927) have found a high degree of protection in rats by feeding it to the females before mating, during pregnancy, and during the first two weeks of lactation.

In applying these principles to the diet of the cow, a difficulty arises from the fact that doses of cod-liver oil large enough to raise the antirachitic value of the milk (6 to 8 ozs.) depress the percentage of butter fat.

A winter diet, containing silage and hay, produces a milk of moderate antirachitic power, but its calcifying properties are less than in summer-produced milk. (The effect of irradiation on winter-produced and summer-produced milk is stated by Supplee and Dow (1927) to be much greater, as regards its antirachitic potency, in the case of winter-produced than summer-produced milk.)

(ii) *Vigantol*.—An interesting prophylactic experiment has been carried out by Eufinger and co-workers (1929). They found that the colostrum or milk from women who had received 10 drops of vigantol daily for several months (with a minimum of 1 month) prior to delivery brought about healing in several rachitic rats, whereas the colostrum or milk from recently delivered healthy women produced no healing of the bony rickets, and the rats died within 10 days after treatment. The mother's milk appears to be just as potent in vitamin D whether the vigantol is given for several months or one day, which points to the conclusion that the vitamin is transferred directly to the milk without any intermediary metabolism. Similar results have been obtained by Guggisberg (1929).

(iii) *Irradiated Yeast*.—Irradiated yeast has been stated by Wachtel (1929) to increase not only the quantity but the vitamin D (and B) content of cows' milk, when fed to the cow.

(iv) *Low Protein Diet*.—According to Krauss (1929), greater Ca deposits resulted from milk produced by cows on low protein diet than from milk made by cows on high protein diet. The same difference was noted when butter fat from these milks served as source of vitamin D.

The results obtained on the question of diet in relation to antirachitic potency of milk by Golding and Zilva (1928), Grant (1926), Kennedy and Palmer (1926), Luce (1924), and others, seem to show that it is possible, by controlling the diet of the cow, to obtain a milk of fairly high antirachitic potency in the winter, and that in the case of human milk the best way of protecting offspring from rickets is to ensure that the mother has a diet containing an ample supply of green vegetables, eggs, milk, and a daily dose of cod-liver oil.

Effect of Maternal Diet on Calcium and Phosphorus Content of Cows' Milk.—While it has been definitely shown that the vitamin D content of the cow's milk can be increased by appropriate feeding and sunlight, and that the retention of calcium and phosphorus is improved, the reports are conflicting as to the effect on the quantity of calcium and phosphorus in the milk and their relationship. The question has been recently investigated by

Sheehey and Brendon (1930). The "normal ration" of the cows, on which the milk remained unchanged for 4 to 5 months, consisted of various meals, corn, and oats, so that $3\frac{1}{2}$ lb. of the mixture contained $2\frac{1}{2}$ lb. of starch equivalent and 0.6 lb. protein equivalent. To this diet for testing purposes were added varying proportions of olive oil and cod-liver oil. Daily determinations of calcium in the milk were made and phosphorus determinations for each period on daily aliquots. No change in the percentage of calcium and phosphorus in the milk was found either when olive oil or cod-liver oil was added to the normal diet, or when olive oil was substituted for cod-liver oil.

(b) **MATERNAL IRRADIATION.**—The same diversity of opinion seems to exist upon the subject of exposure of the mother to irradiation. The results of Steenbock and co-workers are an illustration of the difficulty. In 1925, using goats as the experimental animals, they found that irradiation of the mother practically doubled the antirachitic value of the milk. In their later experiments, however (1930), they state that daily exposure of cows to sunlight or artificially generated ultra-violet radiation has little, if any, effect on the antirachitic potency of the milk. They recognise that summer-produced milk and butter fat are, as stated by Chick and Roscoe (1926) and others, undoubtedly superior to that produced by cows fed on a diet of cereals and roots in the winter, but state that this superiority must have its primary origin in other factors than sunlight acting directly on the cow. A tentative suggestion is made to explain the discrepancy between the 1925 and the 1930 experiments, that cows may differ from man, the goat, the chicken, and probably the rat, in that they are not influenced by short-wave lengths of radiation, and derive their antirachitic vitamin from their food.

Gerstenberger and co-workers (1927) found that exposure of women to ultra-violet light increased the antirachitic potency of their milk, and suggested that the primary cause of the development of human rickets is a deficiency rather of ultra-violet light falling on the skin than of antirachitic vitamin ingested with the food.

Pirquet, however (1927), has reported promising results, both with animals and children, from direct irradiation of the cow. The mercury quartz lamp which provides the irradiation is movably suspended from the roof.

Pasteurised Milk.—It is stated by Schmidt-Nielsen (1929) that pasteurisation of milk at 63° C. has no effect upon its vitamin D content.

Evaporated Milks.—According to Honeywell and co-workers (1930), vacuum evaporation, aeration, and sterilisation tend to decrease the ossifying potency of raw milk.

Ice-Cream.—Irradiated ice-cream, prepared from winter cream, according to Russell, Button, and Kahlenberg (1929), healed rickets in the white rat, whereas the non-irradiated product of the same batch of ice-cream, fed at a maximum level of 8 per cent. of the ration, did not. A variation of the temperature of the product during irradiation between 15.5° and 62.5° C. (60° to 145° F.) did not affect the activity of the material, nor did freezing and low storage temperature affect the antirachitic value of irradiated ice-cream for at least 2 months.

(F) **EGGS**—(1) **Yolk.**—It is a well-established fact that egg-yolk is a good source of antirachitic vitamin. The potency of the yolk has been tested by Hess (1923) both on young rats and infants. Rats were not only protected from but cured of rickets by the addition of 0.5 gram daily, while the yolk of one egg added to the regular diet of each of 12 infants protected them from rickets during the winter ebb of blood phosphate. The percentage of inorganic phosphate in the blood, instead of falling to 3.6 mgrms. (the ebb observed the previous March), was maintained at 4 mgrms. or more, a level encountered during the summer months. The calcium content of the serum also was unusually high. It seems evident, from these data, that yolk of egg possesses considerable protective value in rickets.

The value of egg-yolk in infant feeding has been emphasised by Jex Blake (1927), who regards it as a good source of vitamin D, and considers that egg-water (2 or 3 whole eggs beaten up in a pint of water) might replace the albumen water given to young infants.

The value of egg-yolk in supplementing diets deficient in calcium has been shown by Tso (1926), who has also proved that eggs treated in the Chinese manner, to preserve them as "Pidan," retain their full vitamin D content.

A preparation made from egg-yolk, called heliocitin, is stated by Steudel (1929) to be powerfully antirachitic, both in prophylactic and curative experiments. This protein-free fatty fraction of egg-yolk is rich in lipoids, lecithin, and sterols, and is also a good source of vitamin A.

(2) **White.**—The white of the egg is stated by Hess (1923) to be not only devoid of antirachitic properties but apparently to enhance the rickets-producing quality of the dietary.

FACTORS IN INCREASING THE ANTIRACHITIC POTENCY OF EGGS.—Diet and exposure to light of the hen, according to Bethke, Kennard, and Sassamann (1927), are both effective in producing eggs which contain an adequate amount of vitamin D. Hart, Steenbock, and co-workers (1925) increased the potency tenfold by irradiation of the hens.

(G) **VEGETABLES.**—The amount of vitamin D in green vegetables was at one time supposed to be comparable with their content of vitamin A, but later work has shown that this is not the case. Weinstock (1924) tested the antirachitic potency of fresh lettuce leaves, but found none. Spinach leaves were tested by Goldblatt and Zilva (1923), Zucker and Barnett (1923), Chick and Roscoe (1926), and Boas (1926); they were devoid of antirachitic value except at the height of summer, when a very slight antirachitic effect was found.

Roscoe (1927) demonstrated an effect upon calcification from spinach "as purchased," spinach "sunned" after purchase, and spinach picked at noon after exposure to morning sunlight, the effect, however, being far short of that produced by cod-liver oil. Since no difference in effect could be noted between the three types of spinach, it is concluded that vitamin D is not rapidly removed from spinach in darkness.

Hess and Weinstock (1924), however, found that green leaves, after exposure to artificial ultra-violet light, acquired antirachitic properties, and Chick and Roscoe (1926) confirmed this observation with regard to spinach. Artificially irradiated green spinach given to rats produced as marked an increase in the calcium content of the bones as did giving cod-liver oil, though spinach grown in the summer under the most favourable conditions of natural illumination contained only a very slight amount of the vitamin. No definite explanation is given for the discrepancy between the effect of direct sunlight and artificial irradiation; the suggestion is made that the demonstration of the relatively small antirachitic activity of the green vegetable may be obscured on account of the abundance of vitamin A present in the green leaves. This vitamin A causes rapid growth and, as is well known, the severity of rickets in young animals fed on deficient diets is in general proportional to their rate of growth.

Chick and Roscoe have also put forward the suggestion that in spinach grown in the open the vitamin D may be removed or destroyed shortly after formation, whereas in spinach that has been cut before ultra-violet irradiation no such change can occur.

In experiments on rabbits, Mellanby and Killick (1926) have reported a moderately well-marked antirachitic effect of cabbage, grass (summer grass being considerably more potent than winter grass), swede turnips, and carrots.

According to Steenbock, Hart, and co-workers (1925), clover hay dried in the sun without exposure to excessive weathering has considerable antirachitic potency, while hay dried in the dark, though a fine green colour, has none.

(H) **FRUITS.**—On the whole, fruits are deficient in vitamin D.

(1) **Bananas**, according to Grüninger (1928), contain sufficient to prevent the severer

forms of rickets which develop on an entirely vitamin-D free diet, but Eddy and Kellogg (1927) report them as having little or no antirachitic potency.

(2) **Orange juice**, tested by Willimott (1928) for vitamin D by the Zucker method, failed to change the faecal reaction of a rachitogenic diet to acid, indicating a probable absence of vitamin D.

(3) **Yautia and Platano**.—The yellow yautia and the ripe plátano, products of Porto Rico, have been found by Cook and Rivera (1929) to possess slight antirachitic power, but could not be considered as adequate supplements to a diet of rice and beans, unless some other food containing vitamin D is added to the diet or the individual is exposed to sunlight.

(I) **CEREALS**.—Most cereals are low in antirachitic potency, but the researches of Steenbock (1924) and others have shown that their potency can usually be increased by irradiation (see p. 164).

Rice and beans have been shown by Cook and Rivera (1929) to be almost entirely lacking in vitamin D. Young rats were placed on a basal diet of 60 per cent. boiled rice and 40 per cent. boiled beans (purchased ready cooked from a restaurant and thus containing small amounts of other vegetables, as is the custom in Porto Rico). Growth was poor on this diet, and the rats developed definite signs of rickets. The addition of cod-liver oil to the diet resulted in the growth being almost normal. Exposure of the rats to sunlight improved the growth to about half normal.

(J) **VEGETABLE OILS**.—In their natural state the vegetable oils have not a very high antirachitic potency. According to McCollum, Simmonds, Becker, and Shipley (1922), olive oil, cotton-seed oil, maize, and sesame oil have a moderate curative and preventive action against rickets in a dosage of 10 to 20 per cent. of the ration. When irradiated, their potency is apparently slightly increased (see p. 165).

(K) **CHOCOLATE**.—Reid and Krasso both report experiments with irradiated chocolate.

Reid (1929) states that animals which had been fed with irradiated chocolate showed a greater increase in weight than the control animals, which had been fed with ordinary chocolate, and that harmful effects of irradiated chocolate, as hypervitaminosis, never became manifest.

Krasso (1929) found an increase of appetite in patients fed with 50 grms. daily of irradiated chocolate and two cups of hot milk to which 1 to 3 teaspoonfuls of powdered irradiated chocolate were added. Cases of cachectic phthisis often showed an improvement in their general condition, a gain in weight, and a decrease in the toxic manifestations. Good effects were noted in patients with chronic exudative pulmonary tuberculosis and in those with fibroid phthisis. Favourable results were likewise noted in patients with multiple lymphoma and in one case of enterocolitis. In patients with secondary anæmia an increase was noted in the number of erythrocytes and in the hæmoglobin.

XXXVII. VITAMIN E.

The existence of a specific "antisterility" vitamin, belonging to the class known as fat-soluble, is now definitely established.

For the following account of its nature, distribution, and physiological properties, the writer is chiefly indebted to the extremely comprehensive investigation by Evans and Burr (1927), reported in the *Memoirs of the University of California*, vol. 28.

(A) **OCCURRENCE OF VITAMIN E**.—Unlike the other fat-soluble vitamins, vitamin E does not find its richest source in animal tissues. Cod-liver oil, in particular, which is a fertile source of vitamins A and D, is very low in vitamin E, as also is milk and even egg-yolk. Muscle tissue, animal fats, especially lard, and all viscera, including heart, spleen, kidney, brain, testis, and placenta, have been proved by Evans and Burr to cure sterility only in very large doses.

In the plant kingdom, however, vitamin E is more widely distributed. Green leaves and the germs of certain cereals, particularly wheat, contain it in abundance, and certain oils extracted from these potent substances can therefore be regarded as efficacious in curing sterility in animals, but none to such an extent as wheat-germ oil.

Experiments by Evans and Hoagland (1927) tend to show that vitamin E can be synthesised by the plant from inorganic sources.

(B) **CHEMICAL NATURE OF VITAMIN E.**—The concentration of vitamin E depends upon the fact that its solubility is definitely lipoid in nature. Evans and Burr have extracted the active principle from the golden yellow oil which forms about 10 per cent. of the content of wheat germ. They have used ether, acetone, benzene, and absolute alcohol as extracting agents, the germ being dried at 100° to 110° C. for 2 hours before extracting. The solvents are removed by a water-bath, except the last portion, which is removed *in vacuo*.

(C) **SOLUBILITY OF VITAMIN E.**—(1) **In Water.**—Animal experiments have indicated only a slight solubility in water. The extract obtained when lettuce leaves are shaken for several hours with water has been found to be prophylactic against sterility, but Evans and Burr suggest that this is probably not a true solution but an emulsion.

(2) **In Ether.**—The complete solubility of vitamin E in ether has been established by the fact that the minimum curative dose of the oil (10 per cent. of the germ), extracted by ether from wheat germ, is 25 mgrms. daily, while that of the wheat germ itself is 250 mgrms.

(3) **Acetone and Benzene.**—Solubility in these fat solvents has been found to be practically as complete as in ether (Sure, 1925).

(4) **Alcohol.**—The solubility in alcohol is high, but not complete, falling off rapidly with dilution. Even with hot alcohol some of the vitamin E remains in the residual oil. The fact that the solubility of the E fraction in alcohol is much greater than that of sitosterol has, however, made it possible to separate E from the sterols, and to make concentrated fractions of it.

(D) **STABILITY OF VITAMIN E.**—Vitamin E is remarkably stable to heat, light, air, and chemical reactions generally. Its properties in this respect distinguish it from the other fat-soluble vitamins.

(1) **Heat.**—The vitamin was found to be unaltered in activity by heating for 2 hours at 155° C., stable at 170° C., and only destroyed by ashing at high temperatures (300° to 450° C.). It was also found that the vitamin E in concentrated fractions of wheat-germ oil was not destroyed by distilling *in vacuo* at 250° to 255° C.

(2) **Oxidation.**—Evans and Burr's experiments show that oxidation, by passing air through wheat-germ oil at 97° C., did not entirely destroy the activity of vitamin E.

The destruction of vitamin E, in connection with the rancidity of fats, is considered by Evans and Burr, and by Mattill (1927) an oxidative process.

Evans and Burr (1927) showed that if the amount of lard in the diet is increased (from 8 to 22 per cent.) sterility in first breedings is greatly augmented, and that 10 per cent. of lard, added to a diet containing 5 per cent. of butter, caused a change of high initial fertility (83 per cent.) to one of high sterility (92 per cent.). Contact with lard destroyed the curative action of a minimal effective dose of wheat germ. Stearic acid had no effect, but oleic acid was even more destructive to vitamin E than lard. The oleic acid used had a strong odour and flavour of rancidity, and as it seemed unlikely that pure oleic acid itself was responsible for the effect, lard, butter, wheat-germ oil, and oleic acid were allowed to turn strongly rancid and tested on a fertility diet containing wheat germ. Under these conditions lard and oleic acid increased their power to render vitamin E ineffective, whilst butter and wheat germ not only lost their curative effect but became active antivitamins. This antivitamin action in fats does not run parallel with the free fatty acid number, but definite evidence was found that there is a

great concentration of the antivitamin in the unsaponifiable fraction (4.6 per cent.) of oleic acid.

Mattill further investigated the effect of rancidity, especially in butter and milk fat, and the factors which hastened or delayed the onset of rancidity. He concluded that the apparent protection against oxidative destruction conferred by the presence of vegetable oils, especially wheat-germ oil, is due to the fact that they contain more hydroxy compounds than lard, cod-liver oil, butter, and other animal fats. They therefore delay autoxidation of fat and prevent accompanying destruction of vitamin E. He considers that McCollum's (1927) observations on the connection between vitamin E and iron metabolism (to be referred to later) can be explained on the basis that ferrous sulphate acts by accelerating oxidation of the fat. Similarly, the presence of other oxidising catalysts and anti-oxidisers in various foods must be taken into consideration in testing foods for vitamin E.

(3) **Alkalies.**—The experiments of Evans and Burr in obtaining a non-saponifiable fraction of wheat-germ oil for curing sterility in the rat show that the active principle is entirely stable to alkali under conditions of restricted oxidation. This fraction is 5 per cent. of the oil and is largely sitosterol.

(4) **Acids.**—Vitamin E is remarkably stable towards acids. An extract from wheat-germ oil, emulsified with N/10 HCl and 20 per cent. HCl at room temperature for 34 hours, was found potent. The active substance was also entirely stable to warm, dry, HCl in absolute alcohol. Its activity was, however, entirely destroyed when boiled with acetic anhydride.

(5) **Hydrogenation.**—The active principle in wheat-germ oil is not injured by complete hydrogenation in the presence of palladium in an acid medium.

(6) **Bromination.**—Vitamin E is destroyed by saturation with bromine in glacial acetic acid.

(7) **Ultra-Violet Rays.**—There is partial destruction of the active material after 45 minutes' exposure to a strong quartz mercury lamp.

(8) **Ferric Chloride.**—It is stated by Waddell and Steenbock (1928) that the vitamin E potency of a ration composed of natural and varied food materials may be completely destroyed by treatment with FeCl_3 . The vitamin A content of this Fe-treated ration is not appreciably affected nor is the palatability greatly reduced.

(E) **CONCENTRATION OF VITAMIN E.**—Evans and Burr have attempted the isolation of vitamin E from wheat-germ oil on the same lines as the fractionation of cod-liver oil for vitamin A by Drummond and other workers. They found the active principle to remain in the non-saponifiable fraction of an ether extract (saponified in the cold with 20 per cent. alcoholic KOH). This non-saponifiable matter (5 per cent. of the total 6 kgs. of wheat-germ oil originally used) was further extracted by cold pentane and hot methyl alcohol, then crystallised from the cold methyl-alcohol solution. From a petroleum-ether solution of this crystallised product the sterols were precipitated by digitonin, and the sterol-free oil refluxed in hot 20 per cent. alcoholic KOH, an inactive fraction, containing fatty acids being thus separated from the non-active saponifiable portion. A further precipitation of sterols with digitonin, and a treatment of the resulting sterol-free oil with boiling methyl alcohol yielded an orange solution which proved active in single doses of 5 to 10 mgrms. This has been still further purified by distillation *in vacuo*, and a fraction boiling at 200° to 233° and upwards at 0.5 mm. is found to contain the bulk of the active agent. This portion represents about 5 per cent. of the non-saponifiable matter or 0.25 per cent. of the wheat-germ oil.

Beyond this point further fractionations have been carried out. Removal of sitosterol by pentane and precipitation with alcohol, followed by treatment of the residue with petroleum ether, which is finally removed *in vacuo*, leaves a highly concentrated fraction in which some digitonin-precipitable materials still remain. This fraction has been found to contain no

nitrogen, sulphur, or halogen, and only a trace of ash. Evans and Burr suggest that the active substance is probably an organic compound containing only carbon, hydrogen, and oxygen. The physical properties of a fraction still further purified by treatment with digitonin are those of an orange, viscous oil, hardening at low temperature, but not forming crystals on standing, readily soluble in alcohol, ether, pentane, etc., and insoluble in water and dilute alcohol. All sterol tests are negative. It is not inactivated by hot saponification or by decolorisation by hydrogen in the presence of platinum black or palladium. It is inactivated by bromination and by boiling acetic anhydride. Even the most purified fractions, however, are still too complex to give definite information as to the true nature of the vitamin.

(F) **SPECTROSCOPIC PROPERTIES OF VITAMIN E.**—The absorption spectra of the distillation fractions of the non-saponifiable portion of wheat-germ oil, compared with those of sitosterol, cholesterol, and other sterols, do not point to any definite curves for vitamin E. The distilled vitamin E fraction has, in common with the vitamin A fraction, a coefficient of absorption for all wave-lengths at least one hundred times as great as those for the crystalline sterols.

(G) **REFRACTIVE INDEX OF VITAMIN E.**—The non-crystalline portion of all Evans and Burr's active fractions has an index between $N_{20} = 1.5015$ and 1.5021 . The following table, taken from Evans and Burr, shows an interesting comparison between the various properties of the vitamin E fraction, the vitamin A fraction, as recorded by Drummond and co-workers, and by Takahashi, and cholesterol :

Substance.	Analysis.		Molecular Weight.	Possible Formula.	Iodine No.	Acetyl No.	Refractive Index.	Distilling Temperature. Degrees C.
	C	H						
Cholesterol	83.4	11.9	386	$C_{27}H_{46}O$	65.8	131	...	About 200° C. (10 mm.)
Biosterin (Takahashi) ...	81.0	11.0	402	$C_{27}H_{46}O_2$	180	139	1.52517 (20°)	147° C. (0.02 mm.)
Vitamin A fraction ... (Drummond)	77.7	11.8	300	$C_{20}H_{36}O_2$	124	215	1.4705 (20°)	180–220° C. (1–2 mm.)
Vitamin E fraction ... (Evans & Burr)	81.7	12.2	400	$C_{36}H_{64}O_2$	220	...	1.5009 (20°)	200–233° C. (0.5 mm.)

XXXVIII. PHYSIOLOGICAL ASPECTS OF VITAMIN E DEFICIENCY.

The characteristic effect of vitamin E deficiency is a disturbance of the reproductive function in animals, leading eventually to sterility. The features of this "sterility disease" are quite distinct from the adverse effects upon reproduction produced by any other dietary inadequacy.

In other types of dietary sterility as, for example, in that due to vitamin A deficiency, the disturbance of the reproductive mechanism takes place before implantation occurs, so that gestation does not result. In vitamin E deficiency implantation occurs, preceded by all the preliminary steps, œstrus, copulation, ovulation, fertilisation, and tubal passage. Evans and Burr have proved the invariable occurrence of implantation by the "erythrocyte sign," the appearance of red blood corpuscles in the vaginal canal on the thirteenth, fourteenth, and fifteenth days of gestation. The characteristic ill-effect of vitamin E deficiency, however, now takes place in the form of death and resorption of the developing embryo; so that the young are never born. Evans and Burr have found that in the majority of cases the death of the foetus takes place on the thirteenth day of gestation, but they point out that the time may vary with the type of diet used. Without rigorous purification, minute amounts of vitamin E tend to remain.

(A) **INITIAL FERTILITY.**—When the sterility characteristic of vitamin E does not at first appear in animals fed on a diet from which vitamin E is withheld, these animals are said to exhibit an “initial fertility.” This may be due to incomplete purification of the basal ration. On Evans and Burr’s Standard Diet, consisting of :

Casein	18
Corn starch	54
Lard	15
Milk fat	9
Salts	4
Dried yeast	0.4–0.6 gram

many animals gave birth to a first litter of living young. The removal of milk fat from the dietary reduced the initial fertility from 9 per cent. to 5 per cent. It has been suggested, particularly by V. F. Nelson (1928), that the cause of sterility might be not a vitamin deficiency but a high proportion of fat in the diet. When 24 per cent. of milk fat, however, is present in dietaries the animals show not only an initial but a persistent fertility. The production of sterility by large amounts of lard in the diet is explained, not by its presence as fat *per se*, but by a hypothesis that it contains an “antivitamin E,” in the same way that Mellanby postulates an antivitamin D substance in certain cereals.

This theory is in accordance with the facts stated above regarding the rancidity of fats and its destructive effect upon vitamin E.

Initial fertility may also be due to the storage by the body tissues of vitamin E. Every animal starts life with a considerable store of vitamin E, which receives daily additions from foodstuffs containing it.

This store is only gradually exhausted when vitamin E is withdrawn from the diet, and sufficient may remain to prevent fatal damage to the embryo of a first or even second gestation.

(B) **CHARACTERISTIC ABNORMALITIES IN VITAMIN E DEFICIENCY**—(a) **Female.**—The ovary in the female remains normal in vitamin E deficiency in strong contradistinction to the corresponding changes in the testis in the male. Lack of vitamin E in the female is focused upon the placental site, with consequent intra-uterine failure.

(1) **ABNORMALITIES OF GESTATION.**—Up to the eighth day of gestation the developing egg and the uterine cavity show no deviation from the normal. After that date abnormalities began to appear in the embryo, its membranes, and the placenta.

The Embryo.—The chief feature is a retarded development of the mesoderm, but the establishment of the ectodermal cavity, the formation of the amniotic fold, and the obliteration of the ecto-placental cavity are also much delayed.

The changes in the embryo, which has died *in situ*, or is removed just before death, show a general under-development, together with a deficiency of the blood-forming system. These changes are apparently closely connected with concomitant alterations of the yolk sac and interference with paraplacental nutrition. After death the foetus becomes macerated and finally completely resorbed.

The Membranes.—The yolk sac shows a marked reduction in size and number of the blood islands and villi. The allantois shows atrophy of the stalk, of the foetal blood vessels, and of the villi.

The Placenta.—The only constant changes in the placenta up to the time of foetal death are under-development and an enlargement of the maternal sinuses in the decidua. After the death of the foetus the placenta continues to live and to increase in size for a day or two, afterwards diminishing until about the twentieth day, when coagulation necrosis takes place, followed by resorption.

(2) CAUSE OF DEATH OF THE FŒTUS.—Evans and Burr put forward a "starvation and asphyxia" theory. According to this theory the embryo is starved, owing to the failure of the yolk sac to function as an agent of nutrition, and deprived of oxygen owing to interference with the circulation through the cord.

(3) THE CURE OF VITAMIN E STERILITY IN THE FEMALE.—The abnormality in the reproductive mechanism produced by vitamin E deficiency can be restored to normality by the administration of foods containing vitamin E, either in small daily amounts or in one large dose at the beginning of or up to the fifth day of gestation. The minimal daily dose for the rat has been standardised in the form of wheat-germ oil in a dosage of between 25 to 30 mgrms. daily. This dosage must be begun not later than the fifth day and continued up to the nineteenth day in order to produce living young. The minimal single dose has been found to be 550 mgrms. Either daily amounts or a single one can be given by parenteral injection. Living young have been produced in animals hitherto sterile by the daily intraperitoneal injection of 75 mgrms. of wheat-germ oil, and by 1.1 gram similarly injected as a single dose on or within five days of the beginning of gestation. The question of increasing fertility beyond normal limits by an excess of vitamin E has also been investigated by Evans and Burr. They have found that the reproductive mechanism is not stimulated beyond normal limits even by the administration of foods or extracts of foods known to be twenty times as rich in vitamin E as is necessary for normal reproduction.

(b) Male.—The sterility disease of vitamin E in the male is characterised by a variable period of initial fertility, followed by the ultimate occurrence of sterility, when invariable changes are found in the testes and ejaculate. (In the case of "second and third generation" males, whose mothers have been reared on a vitamin E-free diet, temporary fertility having been induced by highly concentrated extracts of vitamin E, sterility is present from the beginning of sexual maturity.)

The preliminary fertility period may extend from the beginning of the second month to $3\frac{1}{2}$ months of age. From $3\frac{1}{2}$ to about $6\frac{1}{2}$ months spermatozoa are present in various stages of viability in the ejaculation. At the fifth month the majority of animals are sterile, at the eighth or ninth month the spermatozoa disappear, and at the thirteenth or fourteenth month there is loss of power, followed by complete loss of all sex interest.

Loss of sex interest does not take place in the early stages of sterility; even when spermatozoa are no longer emitted with the ejaculatory plug, and when the degenerating testicular epithelium can no longer be rescued by the highest dosage of vitamin E, sexual behaviour tests show entire normality.

(1) CHANGES IN THE EJACULATE AND TESTIS IN VITAMIN E DEFICIENCY—*Ejaculate*.—At first the spermatozoa are normal in structure but are non-motile and, presumably, dead. In the next stage (within a few days) fusion of the spermatozoa takes place, at first possessing their individual heads, and later without heads, but with a considerable cytoplasmic mass at one end. The tightly fused tails of the spermatozoa show a characteristic deeply staining reaction with hæmatoxylin at this stage. Although injury and destruction has thus taken place in the ripe germ elements there is no appreciable structural alteration in the testis at this stage.

Testis.—In the early stages the tubules remain apparently normal. Later (Evans and Burr show sections of testis removed from a rat $7\frac{1}{2}$ months of age on a vitamin E-deficient diet from birth), degeneration of some tubules sets in. In late stages of vitamin E deficiency the testes are small, pigmented watery bodies, weighing less than one-third their normal weight. The majority of the tubules show complete degeneration with loss of cell-layer structure. The age at which this stage is reached varies with the individual animal; in sensitive ones the time required may be less than seven months; while more resistant ones

may not show the typical appearances until near old age, though they are incapable of reproduction in early life. Some tubules, which contain no uninjured epithelium at the time when cure is instituted, continue to degenerate; others, which contain more resistant cells, undergo mitosis, and eventually replace the lost epithelium. Thus the testis of an animal undergoing cure may contain two distinct types of tubules, large ones with more or less normal epithelium, and smaller ones in which only the residual sertoli cells remain.

A further detailed study of the histological degeneration of the testis has been reported by Mason (1929, 1930). Prophylactic administration of small amounts of vitamin E prevents all such histological changes in rats on a diet which, without the inclusion of these small amounts, would bring about the degeneration described above. It is apparent, therefore, that the male germinal tissue is vitally affected by the lack of minute traces of vitamin E. Mason (1926, 1929, 1930) has found changes in the epididymis and in some cases in the prostate and seminal vesicles which appear to be secondary to the degeneration of the testis and not a primary result of vitamin E deficiency. The suprarenals, thyroid, pancreas, liver, spleen, and lymph glands are apparently unaffected; in fact, the effects of lack of vitamin E appears to be confined to the reproductive tract and to be of a highly specific character with respect to fertility. A condition of hydroph testis is described by McCarrison (1930) as occurring in the course of experiments on the relative stone-producing potencies of cereal grains. He suggests that this condition, though it differs from the degeneration of the testis due to vitamin E deficiency in producing a considerable increase instead of a decrease in weight of the organ, may be a late manifestation of vitamin E deficiency.

(2) **CURE OF VITAMIN E STERILITY IN THE MALE.**—It is interesting to note the regeneration of testicular epithelium corresponding to cure of sterility by administration of vitamin E in the form of wheat-germ oil. When cures are instituted late in sterility (about the seventh month) only about one-quarter of these cases regain fertility. At this time there is complete degeneration in from 10 to 90 per cent. of the tubules.

That some of these cases do recover proves that some tubules from which all spermatozoa have disappeared are still capable of regeneration.

Evans and Burr believe that the microscopical appearances of degeneration are no sure criterion of the capacity of the testis for repair.

(C) **OTHER EFFECTS OF VITAMIN E DEFICIENCY**—(1) **Failure of Lactation.**—Sure (1926) has reported experiments in which the lactation of rats receiving 10 per cent. of butter fat in the diet was inferior to that obtained when 1 per cent. of wheat-germ oil was included. Since butter fat is known to produce fertility when given in fairly large amounts (Evans reports 24 per cent., but Sure states that he has ensured continuous fertility with only 10 per cent., and Tso (1927) with 5 per cent.), the question is raised whether vitamin E contains two factors, an antisterility and a galactagogue. Tso reports that daily additions of 10 grms. of fresh lettuce to a synthetic ration resulted in successful lactation.

(2) **Paralysis of Young.**—A spastic and partial paralysis of the body musculature and hind limbs of the suckling young of rats deprived of vitamin E has been found by Evans and Burr to be preventable by wheat-germ oil, yellow corn, lettuce, and all recognised sources of vitamin E. Careful testing of several critical fractions of wheat-germ oil showed a remarkable correspondence between the sterility-curing and the paralysis-preventing properties of these fractions. Since other defects in the maternal diet, such as a high percentage of protein, a calcium or iodine deficiency, and deficiency in vitamins A, B, C, and D were all found to have no effect, Evans and Burr consider that the paralysis is due to a lack of vitamin E.

(3) **Spontaneous Deciduomata.**—Local deciduomatous tumours, such as are sometimes found during pseudo-pregnancy or lactation, have been found by Evans (1928) to occur more frequently in rats on a vitamin E-deficient diet. When tumours were purposely produced

(using silk thread as a foreign body) in rats on diets high and low in vitamin E, the tumours were larger in the E-deficient group.

Evans suggests, therefore, that the absence of vitamin E bears some specific relation to the appearance of these tumours.

(4) **Impairment of Growth and Vigour.**—Rats receiving vitamin E show uniformly better growth and vigour than their littermate brothers or sisters on dietary régimes identical except for the omission of vitamin E. The improvement in growth, due to vitamin E, characterises only the last phase of growth of these animals—the phase after attainment of maturity. The superior growth is obtained when the testes have been removed, indicating that vitamin E does not exert its favourable effects upon growth and the general state of the animal indirectly through its value to the sex gland, but in some other way.

Evans (1928) suggests that the diminution of sex activity of males upon E-free régimes may not be due to impairment of the testes but to the constitutional inferiority of such animals.

A growth factor is also postulated by Mason (1929) who states that both fresh and dried lettuce supplements contain some factor capable of inducing a universally rapid rate of growth as well as an increased growth capacity in male rats.

(D) **STORAGE AND METABOLISM OF VITAMIN E.**—The power of the body tissues to store vitamin E might to some extent be assumed from the existence of "initial fertility." Animals reared on a vitamin E-rich diet do not immediately lose their fertility when transferred to a vitamin E-free diet. Evans and Burr have proved conclusively that the tissues of such fertile animals do contain vitamin E, and that conversely the tissues of sterile animals are depleted of it.

By feeding sterile animals with musculature, subcutaneous and visceral fat, and liver taken from normal animals, they were able to effect cures of sterility. Similar tissues taken from animals of proved sterility, which had been maintained on vitamin E-free diets, failed to effect a cure, even when fed at high levels. They conclude, therefore, that vitamin E is accumulated in the tissues of fertile rats, and is either absent or extremely low in the tissues of infertile rats. They showed also, by feeding to sterile animals newborn young taken from mothers on a vitamin E-rich diet, that the vitamin is always present, though not in high degree, in the tissues of newborn animals. The chief storage place for vitamin E appears to be in the general body musculature and the fat; the spleen, testis, and other viscera are not important storage organs. Experiments were undertaken in order to discover whether vitamin E is used up in the ordinary metabolic processes of the body or whether it is merely a special nutritive requirement for the special function of reproduction. It was found that female animals fed on a vitamin E-free diet, but not allowed to reproduce, were just as infertile at the end of a certain period as those which, on the same rations, had had one or two successful gestations. This evidence would seem to show that vitamin E is used in the ordinary metabolic processes of the body, apart from reproduction.

Vitamin E and Iron Metabolism.—It has been suggested, chiefly on the basis of the experiments of Simmonds, Becker, and McCollum (1927) (since disproved by themselves, 1928), that vitamin E has a specific action in the assimilation of iron. From their results with various diets, containing ferric and ferrous salts respectively, they came to the conclusion that ferric iron had a protective effect against sterility when administered with a rich source of vitamin E, such as wheat-germ oil, and that conversely ferrous iron had a definitely deleterious effect. These results have received a more acceptable explanation than that of a special connection between iron and vitamin E in the hypothesis of Mattill (1927), based upon his experiments with the rancidity of fats.

Mattill considers the destructive effect of ferrous salts to be due to their more rapid oxidative action on fats, the vitamin E in fat being destroyed by the process of oxidation.

Estill and McCollum (1927) have also made an observation which may have some bearing on the relation between vitamin E and FeSO_4 .

They have found that the destruction of vitamin A, which occurs when it is left in contact with FeSO_4 , is inhibited in the presence of a substance which they have separated from cod-liver oil and wheat-germ oil by means of lithium chloride dissolved in pyridine. They suggest that this substance, which tests have proved to be not identical with either vitamin A or vitamin D, may be actually vitamin E. If this is the case it would seem that the oxidative destructive action of FeSO_4 has a stronger affinity for vitamin E than vitamin A. Simmonds and co-workers (1927) have also put forward the suggestion that vitamin E is related to iron assimilation in a manner comparable to the relation of vitamin D to calcium and phosphorus, and that the death of the embryo in vitamin E deficiency is closely connected with a disturbance of iron assimilation. This view has been found to be untenable in the light of Mason's (1929) recent work on the erythrocyte and hæmoglobin content of the blood of vitamin E-deficient animals. Although repeated estimations were made on the same animals at various intervals the erythrocyte and the hæmoglobin content were normal in all cases.

Later work by Simmonds and co-workers (1928) has led them to reverse their former conclusion. They state now that the injurious effect of FeSO_4 on the potency of wheat-germ oil is the result of the destruction of vitamin A and there is no evidence that vitamin E has any rôle in Fe assimilation.

XXXIX. VITAMIN E IN FOODSTUFFS.

(A) **ANIMAL SOURCES.**—Vitamin E is present in a variety of animal tissues, but in low concentration compared with vegetable sources.

(1) **Beef muscle, beef liver, and kidney** contain sufficient of the antisterility vitamin to evoke cures of sterility in a dosage of about 5 grms. daily. Pig tissues were found by Evans and Burr to be inferior in vitamin E content to beef tissues.

(2) **Fat.**—Although lard has been proved devoid of vitamin E, even when present in the proportion of 15 to 24 per cent., fresh unrendered pig fat, as well as fresh beef fat, was rendered efficacious in 5-gram quantities. Simmonds, Becker, and McCollum (1928), however, have reported that certain samples of lard promote fertility to an extent which compels the conclusion that they contain considerable amounts of vitamin E. Milk fat and butter have been reported by Evans and Burr to be very low in vitamin E, 24 per cent. being necessary to produce fertility. Sure (1926, 1927), however, states that with 10 per cent. butter fat, continuous fertility and good lactation were ensured, and Mattill (1927) states that if oxidative changes be retarded, comparatively small amounts of butter fat suffice for normal reproduction.

Milk fat has also been found adequate for the rearing of young rats by Schultz, Ottokarl, and Maurmann (1928), and butter fat 5 per cent., with lard 1 per cent. by Tso (1927).

(3) **Cod-Liver Oil.**—The difference in distribution of vitamin E from the two other fat-soluble vitamins, A and D, is well exemplified in its low proportion in cod-liver oil. The oil from some sources, in Evans and Burr's experiments, occasionally led to the birth of living young when fed at levels of 24 per cent. of the ration, but Sure found 920 mgrms. daily ineffectual, even in an oil known to produce good growth in a dosage of 1 to 2 mgrms. and to prevent rickets in a dosage of 7 mgrms. daily.

Nelson and co-workers (1928), however, have obtained reproduction on 1 per cent. of cod-liver oil (Squibb) and up to the seventh generation on 5 per cent. Simmonds, Becker, and McCollum (1928) have also found certain samples potent. Both Evans (1928) and Sure

(1927) have reported complete inhibition of reproduction when large amounts of cod-liver oil are administered. They believe this action to be a toxic one, due to some component of the oil.

(4) **Milk.**—Milk is low in vitamin E. Evans and Burr maintain that the successful rearing of first litter of offspring is not due to the vitamin E in the maternal milk but to the stores received by them through the maternal placenta during intra-uterine life.

They have found skim-milk powder, unless used in the proportion of one-third of the diet, inadequate to prevent sterility. If the milk is taken from cattle on green pasturage, however, a slight improvement in reproduction results. By adding 5 per cent. distilled water to a milk-powder diet, and administering cod-liver oil separately, Anderegg and Nelson (1926) have claimed to increase its nutritive value sufficiently to have secured fourth generation young, but Sure (1926) has failed to find any sign of fertility in rats on this régime.

(5) **Egg-Yolk.**—One-third of an egg-yolk daily has been found to ensure fertility in rats.

(B) **VEGETABLE SOURCES.**—Even the richest animal sources of vitamin E are low in its content compared with its concentration in certain green leaves and seeds.

(1) **Lettuce.**—Fresh green lettuce leaves have proved surprisingly potent, being curative at a level of 2.5 grms. daily. Dried lettuce leaves, desiccated at a temperature not exceeding 100° C., were efficacious even in doses of a quarter of a gram. Mason (1929) states, however, that lettuce loses a certain amount of its vitamin E content in the drying process, the dried substance being approximately one-eighth as potent as the fresh green material. When extracted with ether, 23 mgrms. daily of the resultant oil were sufficient to ensure normal gestation.

(2) **Alfalfa.**—Alfalfa hay, carefully dried away from direct exposure to the sun, was milled into a powder which ensured fertility in a daily dosage of 0.6 gram.

(3) **Bryophyllum**, and even dried tea leaves, at a level of 2.5 grms. were found to result in the birth of living young.

(C) **CEREALS AND SEEDS**—(1) **Wheat Germ.**—There is no other naturally desiccated substance so rich in vitamin E. When the pure flakes are fed to sterile animals, 250 mgrms. daily are sufficient to effect a cure. Even after a fairly exhaustive extraction with alcohol and ether, the residue, in the proportion of one-third of the diet, is efficacious. A product called "Vitavose," in which the wheat germ has been extracted with benzene, and a residue in which the extraction has been still more completely effected under pressure, both proved ineffective to cure sterility.

(2) Corn germ, alfalfa seed, and lettuce seed have been efficacious in doses of 1 to 2 grms. daily.

(3) Canadian field peas, grown in various culture solutions, and administered in the proportion of 4 grms. daily, resulted in fertility.

(4) Raw peanuts have been employed successfully in 1 gram quantities daily.

(D) **VEGETABLE OILS.**—(1) **Wheat germ oil** is by far the most potent in the antisterility factor. No other vegetable oil is comparable with it in efficacy. Its prophylactic and curative properties in the sterility due to a vitamin E-deficient diet have been amply demonstrated by Evans and Burr in their very large number of experiments. Additional evidence of its specificity is afforded by the observations of Daniels and Jordan (1928) on the oxidation of cod-liver oil. It is known that a certain minimum of vitamin A is necessary for reproduction, and it has been shown by several workers that wheat-germ oil retards the destruction of vitamin A in cod-liver oil. Daniels and Jordan carried out experiments to determine whether wheat-germ oil is effective as an antisterility agent because of the presence of a specific vitamin or because of its retarding influence on the oxidation of vitamin A. Three

groups of rats, each group consisting of 2 males and 4 females, were maintained for a period of 7 months on the following diets :

Diet A	...	Casein	22 grms.
		Corn starch	100 grms.
		Yeast	7 grms.
		Salt mixture	8.5 grms.
(Vitamins A and D were supplied in the form of Oscodol tablets, two tablets, equivalent to 5 per cent. of the ration of cod-liver oil, to every 488 calories of food.)										
Diet B	...	Similar to A, with 3 per cent. added wheat-germ oil.								
Diet C	...	Similar to A, with 12 grms. lard substituted for 27 grms. corn starch.								

Only 1 female in Group A reproduced. In Group B each female produced 2 litters within the 7 months (1 female produced 3 litters). In Group C the results were slightly better than A but in no way comparable with B. Each female reproduced once, but 3 of the litters died within twenty-four hours of birth. These experiments show that the effectiveness of wheat-germ oil as an antisterility agent is due to the presence of some substance (vitamin E) and not to any retarding action which this may have on the oxidation of vitamin A.

(2) **Other Vegetable Oils.**—Coco-nut oil, cotton-seed oil, and linseed oil, however, are efficacious in dosages of 15 per cent. of the ration, and cotton-seed oil in its hydrogenated form (Crisco) and walnut-seed oil in dosages of 22 per cent. Evans and Burr believe that corn oil, olive oil, peanut oil, and flax-seed oil would ensure fertility if they could be given in amounts which would represent the total fat of the ration, while Sure (1926) states that cotton-seed and crude corn oils furnish a sufficiency of vitamin E when forming 5 per cent. of the ration. Lettuce-seed oil is efficacious at a level of 160 mgrms. of the oil daily, representing $\frac{1}{2}$ gram of the seed, and lettuce-seed oil is so potent that a single drop daily is sufficient to effect cures.

(E) **FRUITS.**—(1) **Bananas** confer fertility in a dosage of one-third of a banana daily.

(2) **Orange juice** is variable in its effect, being potent with animals which are sensitive to minimal amounts of vitamin E.

PART III

THE WATER-SOLUBLE VITAMINS

PART III

THE WATER-SOLUBLE VITAMINS (VITAMINS B AND C)

XL. THE VITAMIN B COMPLEX.

Since 1926, when Goldberger and his co-workers demonstrated the existence of two separate factors in the complex hitherto known as water-soluble vitamin B, much of the accepted work in connection with vitamin B has had to be reinvestigated. Many of the facts published and confirmed are now known to be relevant only to the antineuritic, thermolabile factor, B₁, while B₂, the antipellagric, growth-promoting, thermostable factor, remains to be completely investigated. The difference between the pigeon and the rat, the two species chiefly used in all vitamin B experiments, in their requirements of the various factors in the complex, has led to much confusion.

A third factor, growth-promoting, but thermolabile, and necessary for the pigeon but not for the rat, has been described by Williams and Waterman (1928).

A fourth factor, alkali-thermolabile, but present in fresh marmite, which does not contain the Williams and Waterman factor, has been described by Reader (1928, 1929, 1930). The French workers, Randoin and Lecoq (1929), distinguish four factors—(1) antineuritic; (2) “nutritive”; (3) “cellular” (a micro-organism factor); and (4) antipellagric. The first two are alkali-labile and necessary for pigeon nutrition. Peters (1930) considers that the Williams and Waterman factor is distinct from both of these, but that the extra alkali-labile factor may be identical with that described by Reader.

Reader (1930) obtains her B₃ preparation from the filtrate of the preparation of vitamin B₁ by precipitating with mercuric sulphate. Administration of this preparation daily by the mouth was found to cause resumption of growth when no further response had been produced by addition of B₁ or B₂, or both. The suggestion is offered that the vitamin B₃ factor forms an insoluble mercury salt with mercuric sulphate. The growth curve is also stated to indicate that the storage period for vitamin B₃ is approximately five weeks compared to three weeks with vitamin B₂. The following table, drawn up by Peters in his second *Harben Lecture* (1930), summarises what is known of the factors of the B complex, excluding the fifth factor of Coward, Key, and Morgan (see below):

THE “B” COMPLEX.

A. Factors for which evidence is complete.

	Needed by		
	Growing Rat.	Adult Pigeon.	Distinctive Property.
(1) Antineuritic proper (B ₁) Eijkman (3)	yes	yes	alkalilabile
(2) Antidermatitis (B ₂) (Goldberger <i>et al.</i>)	yes	no ⁽¹⁾	alkalitable
(3) Third rat factor (B ₃) (Reader)... ..	yes	probably (2) yes	alkalilabile
(4) Third pigeon factor (Williams and Waterman) ...	no	yes	thermolabile

NOTES.—(1) When pigeons are living upon an adult diet.

(2) Provisionally identified with the rat factor. Probably the same as Randoin and Lecoq “d’utilisation nutritive.”

(3) Oryzanin (rice), torulin (yeast).

(It should be noted that Williams and Eddy (1928) refer to the Williams and Waterman factor as B_3 , and to a further rat factor, which Peters thinks may be identical with the B_3 , described by Reader as B_4 .) Chick and Roscoe (1930) state, on the basis of their experiments, on the sensitiveness to alkali of vitamin B, that the assumption of a third thermo-alkalilabile factor in yeast is unjustifiable. A possible fifth factor is postulated by Coward, Key, and Morgan (1929), thermostable, and necessary for the rat, and present in "light-white casein." This substance restored growth to animals which had ceased to grow on a diet hitherto considered to be satisfactory in calorific value, salt content, and digestibility, and containing an abundance of all recognised vitamins. The basal diet of these animals had been made up with "vitamin-free casein" (Glaxo). Other substances which brought about rapid resumption of growth were fresh milk, lettuce, fresh and dried grass, ox muscle, liver and wheat embryo. Less rapid growth was brought about by watercress and by milk which had been simmered for 15 minutes. Butter and etiolated wheat shoots showed very little influence on the growth of these rats. The power to cause growth resumption was removed at least partially from "light-white casein" by heat, but not by treatment with cold alcohol, ether, or 2 per cent. acetic acid. Extraction with boiling alcohol or with boiling ether partially destroyed this property of casein, and the extracts were slightly active. Boiling alcohol (90 per cent.) and ether gave extracts of wheat embryo of definite activity and left the wheat embryo with only slight activity. Coward, Key, and Morgan suggest that these results indicate either the existence of a growth-promoting factor hitherto unrecognised or a biological inadequacy of the protein supplied to the rats in the basal diet. The preparation of active alcohol and ether extracts supports the former view.

Most of the existing knowledge regarding the distribution of what was called vitamin B has been compiled chiefly on the results of experiments upon the prevention and cure of polyneuritis in pigeons and, therefore, has reference only to distribution of vitamin B_1 .

Although the two chief factors in the vitamin B complex are usually referred to as "antineuritic" and "antipellagric, growth-promoting," Chick and Roscoe (1928) point out that B_2 is not a growth factor in contradistinction to B_1 , since no growth occurs if either is lacking.

XLI. EVIDENCE OF THE DUAL NATURE OF VITAMIN B.

Although to Goldberger and co-workers must be attributed the definite establishment of two separate factors in the B-vitamin complex, earlier workers in the same field had already foreshadowed its duality.

In 1912 Cooper showed that pigeons need a factor in their diet different from the antineuritic substance hitherto regarded as the sole constituent of vitamin B. In 1926 Seidell, following the experiments of Funk, which had shown that the treatment of yeast with adsorbing agents produces two factors, neither of which alone produces growth, attempted to isolate the concentrate from yeast extract. He found that fuller's earth adsorbed a factor which was powerfully antineuritic for pigeons, but did not replace yeast in the normal growth of rats. Seidell then emphasised the existence of two factors from a chemical standpoint.

In 1926 also Smith and Hendrick, in the course of an investigation into the antineuritic properties of the oat kernel, discovered that a diet containing fat, starch, vitamin A, and salts, together with 40 per cent. of rolled oats, was in some way deficient in growth-promoting power. Believing that the oats did not contain sufficient vitamin B (antineuritic), they added 2 per cent. of dried brewers' yeast, when much better growth occurred. They then discovered that the same beneficial effect was obtained if the yeast was autoclaved for 6 hours at 15 lb. pressure—a process known to destroy its vitamin B (antineuritic) activity. Moreover, they found that while the addition of a small quantity of Seidell's antineuritic concentrate to a B-deficient diet was not sufficient to maintain growth, the additional inclusion of autoclaved yeast caused

growth to be resumed. They concluded, therefore, that dried brewers' yeast must contain some factor essential to nutrition other than vitamin B (antineuritic), and that this unknown factor was thermostable. Simultaneous experiments on the growth and development of chicks by Hauge and Carrick (1926) now led them to the conclusion that the water-soluble antineuritic and the water-soluble, growth-promoting vitamins were not identical. A certain yeast was found to be very rich in the growth-promoting substance but poor in the antineuritic, while corn was rich in the antineuritic and poor in the growth-promoting. Two factors were therefore postulated as necessary for growth, a thermostable factor present in autoclaved yeast, and a thermolabile one present in wheat germ.

Scotti-Foglieni (1926) confirmed the existence of these factors and their reactions to heat by testing the antineuritic potency of the two fractions in brewers' yeast. He found the test positive for that fraction which was destroyed by heat, and negative for the substance which remained after subjection to high temperature.

Meanwhile Goldberger and his colleagues had been working on the subject of pellagra, and had reached the conclusion that this disease was caused by the deficiency of some essential food factor which in some respects had similar properties to those of vitamin B, but which was quite distinct from the factor which cured experimental polyneuritis. Neither of the factors alone sufficed to promote growth in young rats, but the two together adequately supplemented a ration deficient in vitamin B, and they therefore concluded that vitamin B consisted of two separate factors. Goldberger was able to demonstrate the antipellagric factor by using an alcoholic extract of corn meal, which proved relatively poor in vitamin B₂ but rich in vitamin B₁.

Sherman and Axtmayer (1927) worked on similar lines. In their experiments the test feeds were fed both separately and together as sources of vitamin B. Better growth was obtained by a mixture of the foods than by double the amount of each given separately; it was concluded, therefore, that the better growth was due to a supplementation of the separate factors of vitamin B. They were able to show this supplemental relationship with ground or whole wheat and autoclaved yeast, also between ground, whole, and dried skimmed milk, but not between autoclaved yeast and dried skimmed milk. These experiments proved that yeast, when autoclaved, is rich in the growth-promoting factor, as also is dried skimmed milk, whereas ground whole wheat is richer in the antineuritic than the growth-promoting factor.

The nomenclature of the two factors at this time caused confusion. Goldberger suggested that the thermostable factor should be named P-P (pellagra-preventing) on the assumption that it was identical in properties and distribution with the factor that he considered as a specific cure for human pellagra. The thermolabile or antineuritic factor could then either retain the old name of "B" or receive the designation of B-P (beri-beri-preventing). Other American workers, for instance Sherman and Axtmayer, have preferred to discard the use of the letter B, and have named the antineuritic factor vitamin F and the thermostable factor vitamin G. In Britain the recommendation was made by the Accessory Food Factor Committee of the Medical Research Council and Lister Institute to use the term vitamin B₁ to denote the antineuritic factor, and vitamin B₂ to denote the thermostable factor.

It should be noted that the thermostable factor is only relatively thermostable. Williams and Eddy (1928) have found that if autoclaving is allowed to proceed too long, the antipellagric potency of autoclaved yeast will also disappear. Vitamin B₂ is then capable of heat destruction but far more resistant to it than vitamin B₁.

The relation between "pellagrous" symptoms and vitamin B deficiency was now further confirmed by Chick and Roscoe (1927). Roscoe found that young rats, fed on a diet deficient in vitamin B, collapsed with neuritic symptoms about the twenty-fourth day; the collapse

could be cured by alcoholic extract of yeast. If the rats continued on the same diet, together with the addition of the alcoholic extract, a fresh and very severe syndrome of "pellagrous" symptoms made its appearance. These symptoms, which consisted of loss of hair and œdema, yielded to the administration of yeast autoclaved at 120 ° C. for 5 hours, which had no effect at all upon the neuritic symptoms.

A fraction which they considered identical, or associated with the pellagra-preventing factor of Goldberger, was isolated by Salmon and co-workers (1928) from the leaves and seeds of the velvet bean, soy-bean, and rape. Salmon (1927) treated extracts of these materials with fuller's earth and found that the fraction adsorbed prevented experimental beri-beri or polyneuritis in pigeons and rats, but did not induce growth in rats. The other fraction had almost no beri-beri-preventing action, and very little growth-promoting action when fed alone, but showed marked growth-promoting action when added to the antineuritic fraction. It was also found that while the seeds contained, weight for weight, more of the antineuritic factor than the leaves, yet the leaves were more potent than the seeds in promoting growth.

A further differentiation of the two factors in yeast was made by Williams and Waterman (1929), who removed the antineuritic factor from yeast, and then found that it failed to maintain growth unless supplemented by yeast which had been heated for 6 hours at 125° C. Similar results have been obtained by Randoin and Lecoq (1928) using a cold 70 per cent. alcohol extract of yeast as their starting-point. Another method of separating the two factors has been suggested by Hogan and Hunter (1928). They have shown that sources of vitamin B when exposed to ultra-violet rays for 10 hours retain their antineuritic powers but lose their growth-promoting properties.

Autoclaving similar sources of vitamin B produces the reverse effect, *i.e.* the heated material lost its antineuritic but not its growth-promoting potency. Neither fraction alone, as the sole source of vitamin B, would permit rats or pigeons to survive longer than a few weeks. A mixture of the two supplements, in the amounts used, was apparently as active as the untreated material.

An almost pure antineuritic concentrate has been prepared by Evans and Burr (1928) from an extract of white rice polishings. This extract, called tiki-tiki, is practically devoid of the growth-promoting factor, and can be used in testing for the presence of vitamin B₂.

VITAMIN B

In the following account of the distribution, properties, etc., of vitamin B₁, it is possible that many of the facts refer to the vitamin B complex. Until further investigation has differentiated the two factors, however, it is thought best to consider all information which has not been specially examined with relation to vitamin B₂ as referring to the antineuritic vitamin.

According to Peters (1929), B₁ or "torulin" is generally necessary for the growth of all cells—rats, pigeons, bacteria, and tissue cultures; yeast cells can make it for themselves.

XLII. OCCURRENCE OF VITAMIN B₁.

(A) IN TISSUES.—The antineuritic vitamin is found in vegetable tissues in connection with the growth capacity of the cells. It cannot apparently be synthesised by animal tissues. Randoin and Simonnet (1927) remark that it is found wherever cells are in a state of activity, or have been in such a state, since vitamin B does not disappear with the death of the cell. Its connection with growth is emphasised also by Burrows and co-workers (1926). They believe that the body under ordinary conditions does not supply sufficient growth stimulus for its own growth and other activities, and that the stimulus must be supplied from other

growing cells in nature. They produce evidence which they state proves that vitamin B is itself this growth stimulus of other cells in nature. At any rate, vitamin B is not as a rule found in any of the tissues whose function is that of storage and maintenance. Such tissue might be considered to be exemplified in the pancreas, but insulin has been shown by Stucky (1928) to be markedly deficient in both the polyneuritic and the growth factors. Abnormal tissue, in the shape of chicken sarcoma, has also been proved by Nakahara and Somekawa (1928, 1929) not to produce or contain vitamin B, and their later reports have shown that the vitamin B in the body of the host is not utilised by the growing sarcoma.

(B) **IN YEAST.**—Its occurrence in yeast, a unicellular organism, is an example of the universality of its occurrence in growing tissue. Most workers consider that yeast can synthesise vitamin B₁ from the medium in which it grows, though not so easily as the growth-promoting factor. Thus Nelson, Fulmer, and Cessner (1921) were able to prevent symptoms of vitamin B deficiency in rats by yeast grown in a synthetic medium, and Harden and Zilva (1921) to cure polyneuritis in pigeons.

Randoin and Lecoq (1926) found that yeast cultivated on cane-sugar, molasses contained less of the antineuritic factor than yeast grown on beet-sugar molasses.

Hawking (1927) grew yeast in a medium containing purified salts, cane sugar, and "Bios extract." Cultures made from the supernatant fluid after incubation showed that only slight bacterial contamination had occurred. The bios extract had been prepared from bakers' yeast and was proved to contain a certain amount of the antineuritic vitamin. The yeast grown for 5 days in the artificial medium contained rather more of the antineuritic vitamin than did the original bios extract.

Hawking's experiments were repeated and confirmed by Peskett (1927), taking special precautions to avoid bacterial contamination. An experiment is recorded in which 5.7 grms. of yeast were produced in a medium containing not more than 0.7 day doses of antineuritic vitamin. By boiling this yeast for 1 minute with 1 per cent. acetic acid, an extract was obtained which, when fed in equal amounts to 2 pigeons, cured symptoms of head retraction, in 1 case for 2½ days, and in the other for 3½ days. The yeast therefore contained at least 6-day doses of antineuritic vitamin, a considerable synthesis of the vitamin having thus occurred.

Vitamin B₁ and "Bios."—The analogy between the bioses that stimulate the growth of yeast and the vitamins that stimulate the growth of higher organisms has been and is still under investigation by many workers. Wildier's work in 1901 on the substance which he termed "bios," and which he considered necessary for the growth of yeast, was the starting-point of much discussion, and many workers suggested that Wildier's "bios" might be identical with vitamin B₁. Another explanation of its action was put forward by Pringsheim (1906) to the effect that Wildier's bios was simply a suitable source of nitrogen, at the expense of which the yeast could grow, until it had been adapted to the synthetic medium. That this might be its mode of action was supported by the results obtained by Wright (1921), who found that yeast cells could grow at the expense of small amounts of yeast extract added to a synthetic medium until a certain concentration of cells was obtained, after which it continued to grow at the expense of the nitrogen of the ammonium salt present. A crystalline amino-acid that seemed to function as a bios was isolated by Kerr, Eddy, and Williams (1924) from yeast and called bios 223. The activity of this compound could be completely blocked by substituting for one of its NH hydrogens an additional compound (benzene sulphon chloride), and restored by removing the substituted product and replacing the hydrogen by means of hydrolysis.

Williams (1919, 1920) had already attempted a quantitative estimation of vitamin B by measuring the amount of growth of yeast in media containing vitamin B. He at first estimated the growth of a single cell by the drop culture method of Lindner, but later

weighed the amount of yeast produced. Bachmann (1919) made a similar attempt, using the amount of CO_2 evolved after a given time as the criterion, and other methods have been devised for measuring the amount of growth by other workers, including Emmett and Stockholm (1920) and Funk and Dubin (1920). Williams followed up the earlier work on bios 223, and was able to separate from yeast a product with the same physical and chemical constants. On testing the activity of this product on yeast he found that the isolated amino acid was not so active as the yeast autolysate from which it was separated, and that its stimulative power was confined to certain strains of yeast. These results showed that the isolated bios was either not the sole bios present in the yeast or else that there was left behind after its isolation some

factor which would produce maximum stimulation, and they are in accord with those of Clark (1922) and Funk and Paton (1922), who found that yeast removes the growth stimulant (bios) from the medium in which it is growing, but does not take up the vitamin B. In this way the two can be separated, showing that the stimulation produced is in all probability by a substance different from vitamin B. Funk and Dubin (1921) and Freedman and Funk (1922) claim in fact to have separated the growth stimulant by fractional adsorption with fuller's earth from the accompanying vitamin B.

Eddy (1928) suggests that these bioses are not growth catalysts but true yeast foods, and that a knowledge of their nature and behaviour may lead to the isolation and chemical reconstruction of the vitamins themselves.

(C) **IN PLANTS.**—In the plant, vitamin B is located in the seed and not in the young shoots or roots. In the case of cereals the largest deposit of an antineuritic factor has been shown by Chick and Hume (1917) to be found in the embryo or germ. In wheat the embryo contains about one-sixth of the total amount in the grain. The bran (pericarp and aleurone layer) contains a certain amount, while the endosperm, when deprived of the aleurone layer by "milling" in the case of wheat, and "polishing" in the case of rice, is practically devoid of vitamin B_1 .

It is interesting to note that the biological value of the proteins of the various layers of the cereal grains appears to run parallel with their vitamin content. The fractions from pericarp and germ, as tested by Klein, Funk, and co-workers (1926), proved superior in nutritive value to those from endosperm.

Vitamin B_1 and Germination.—The fact that vitamin B is present in seeds, but not in the roots or shoots of the young plants growing from them, has led to investigations into the question of the fate of the vitamin during the process of germination.

Kucera (1928, 1929) found that vitamin B disappeared with greatest rapidity during the germination of rye, the vitamin being absent after six days' germination. In wheat and barley it disappeared more slowly, being absent only after 18 days, while in the case of oats

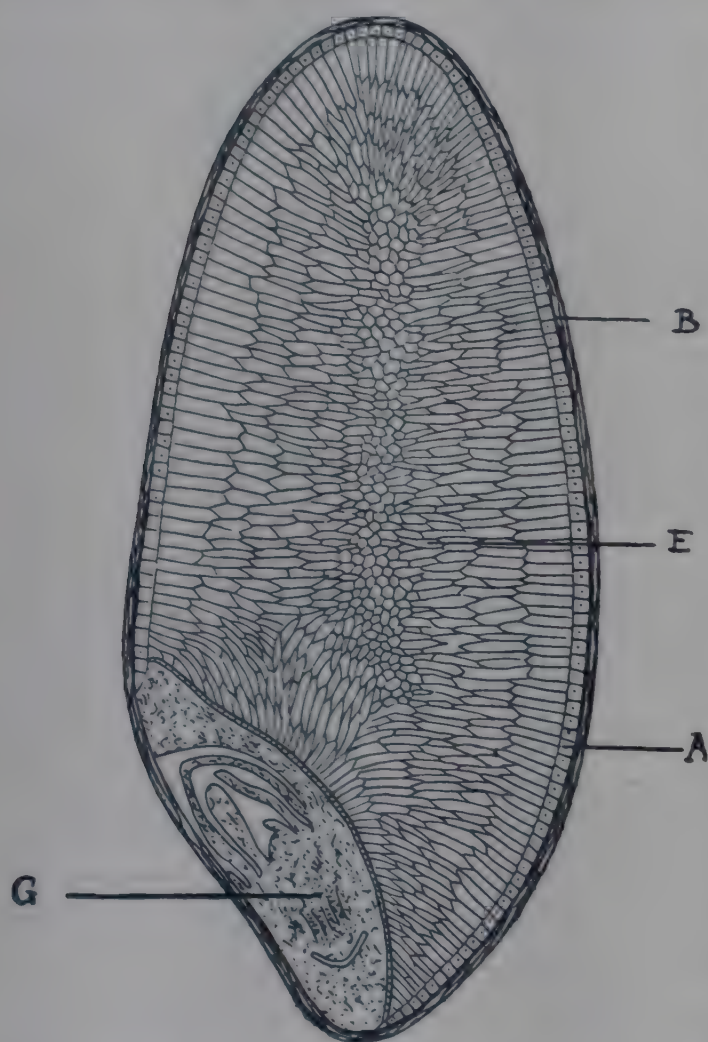


Diagram of a longitudinal section through a grain of wheat, showing: *B*, pericarp, forming the branny envelope; *A*, aleurone layer of cells forming the outermost layer of the endosperm removed with the pericarp during milling; *E*, parenchymatous cells of the endosperm; *G*, embryo or germ.

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and maize some was still present after germination for 18 days. In leguminosæ the disappearance was much slower than from cereals, 15 per cent. of the vitamin B being present after germination for 21 days.

Similar results have been obtained by Hlavaty (1929) from his studies on the germination of the pea and vetch. The disappearance of the vitamin B was apparently accelerated by germinating in the sun instead of in the malting-house. The vitamin B content of both pea and vetch germinated in the sun appeared to be slightly greater after 30 days than after 27 days, indicating that the vitamin never disappears completely during germination, but begins to increase again in the young plant.

Heller's experiments (1928) on the effect of light on the synthesis of vitamins also indicate that the formation of vitamin B is not increased by germination but comes at a later period in the development of the plant.

XLIII. SYNTHESIS OF VITAMIN B₁ BY BACTERIA.

The question of the formation of vitamin B during the growth of bacteria has been discussed in the section "Vitamins and Bacterial Growth," but it has an important bearing on the source of vitamin B present in milk. Human milk has been shown to be often decidedly inferior to cows' milk in its content of vitamin B. The investigations of Bechdel, Honeywell, Dutcher, and Knutsen (1928) have proved that the vitamin B in milk is not dependent on the presence of this vitamin in the ration of the cow, and that a calf will grow normally to maturity, and produce normal offspring on a ration that carried an insufficient amount of the vitamin B complex to support growth and well-being in rats. The results of the studies of these workers have established the fact that vitamin B can be synthesised by bacterial fermentation in the rumen of the cow. Not only were alcoholic extracts of the fermented contents of the rumen proved potent in maintaining and restoring growths in rats, but the predominant bacteria (of the genus *Flavo bacterium*) were also tested and found to contain appreciable amounts of the vitamin B complex. This phenomenon is probably of the same nature as that occurring in "refection," which will be described later.

XLIV. CHEMICAL NATURE OF VITAMIN B₁.

In the earlier days of Funk's work (1911, 1912) on the water-soluble vitamins, he attempted to establish the chemical identity of the antineuritic vitamin with the class of pyrimidine bases. Funk's early observation that many purin derivatives have curative effects on the polyneuritis of pigeons have been verified by other workers, and it has been pointed out by Plimmer (1928) that all the substances so far isolated as vitamin B belong to the group of nucleic acids.

Williams (1917) considered it probable that vitamin B was a cyclic nitrogen derivative capable of existing in two tautomeric forms, one of which is active but readily passes into the more stable inactive form, but he has not been able to bring forward definite proof of his opinion.

Peters' (1929) recent work on torulin has led him to believe that vitamin B₁ is a tertiary base. That it is not a primary one, and probably not a secondary, was shown indirectly by McCollum and Simmonds (1918). A yeast concentrate would still support the growth of rats after treatment with nitrous acid. The fact that nitrous acid does not, as shown by Peters (1924), destroy the antineuritic properties of yeast extract would seem to exclude vitamin B₁ from the group of primary amines. It should be mentioned, however, that Drummond's recent investigations have led him to deny Peters' conclusions and to state that vitamin B₁ is inactivated by nitrous acid.

Peters' view that torulin is a base is also supported by his adsorption experiments. A property of bases is their adsorption by charcoal at alkaline reactions, and Kinnersley, Phelps,

and Peters' experiments (1929) have shown that even the better torulin preparations are adsorbed by charcoal at approximately pH 9.0.

Sahashi (1928) has recently claimed that 2 to 6 di-OH quinoline and also 4 (or 5) glyoxaline ethyl methyl carbinol have curative properties. The latter substance is somewhat similar to a possible formula for Jansen and Donath's compound. Dr. J. M. Gulland has recently prepared these compounds, and Peters and co-workers have tested them upon the pigeon (1929). The compounds were not curative and did not behave like torulin.

Attempts to isolate vitamin B₁ have, however, led to its concentration in a very high degree, the preparation of Kinnersley and Peters (1928) being now curative in the very low dosage of 0.027 mgrm. per day.

XLV. CONCENTRATION OF VITAMIN B₁.

The methods employed in the concentration of the antineuritic vitamin are based chiefly upon its properties with regard to solubility, adsorption, and dialysis.

(A) **EXTRACTION.**—The first step consists in the extraction of substances containing vitamin B₁ by water or dilute alcohol either neutral or slightly acid and in the warm. Methyl alcohol, propyl alcohol, dilute acetone, and glacial acetic acid have also been used as extracting agents. The most commonly used solvent is 70 per cent. alcohol, and, if used in the cold, the extraction must be repeated several times. Péneau and Simonnet (1921) report that 13 such extractions were necessary to remove all the vitamin B from brewers' yeast.

(B) **AUTOLYSIS AND ACID HYDROLYSIS.**—The products of autolysis and acid hydrolysis, according to Funk and Dubin (1921), contain more vitamin than extracts made by various solvents.

(C) **PURIFICATION.**—Proteids and lipoids are removed by dialysis, extraction with ether or dilute acetone.

(D) **PRECIPITATION.**—(1) *With the object of removing inert material.* This may be accomplished by acidification of the watery extract by hydrochloric or sulphuric acid; treatment with 10 per cent. alcohol; precipitation with neutral lead acetate, which carries down very little of the antineuritic factor; and the action of heat in an acid medium.

(2) *With the object of concentrating Vitamin B₁ in the precipitate.* This is accomplished by adsorption on various reagents such as fuller's earth, animal or vegetable charcoal, colloidal arsenic, colloidal iron, etc.

(E) **STEPS IN CONCENTRATION OF VITAMIN B₁**—(1) **Funk's Method.**—Funk and Cooper in 1911 precipitated the curative substance present in rice polishings by phosphotungstic acid as follows:

Rice polishings were extracted with cold absolute alcohol which was partially saturated with gaseous HCl. The extracts were evaporated *in vacuo* at a low temperature, and the fatty residue melted and extracted with water. These aqueous extracts were precipitated with 50 per cent. phosphotungstic acid solution after addition of sulphuric acid to the extent of 5 per cent. and the precipitates decomposed in the usual way with baryta. The solution, entirely freed of baryta and sulphuric acid, was filtered and the filtrate neutralised with HCl and evaporated *in vacuo*. The residue was extracted with alcohol and the solution freed by filtration from the inorganic chlorides. The alcohol solution was then precipitated with alcoholic mercuric chloride solution. The active substance was found to a small extent in this precipitate but the bulk was in the filtrate. From each of these fractions vitamin could be completely thrown down by use of silver nitrate and baryta. From this fraction, after decomposition with hydrogen sulphide, there was isolated a very small quantity of a crystalline substance with a melting-point of 233° C. to which Funk gave the formula C₁₇H₂₀O₇N₂. This was not recrystallised and possessed very marked curative power.

This substance was afterwards shown to be impure *nicotinic acid*.

Funk extended his study of the phosphotungstic precipitate, and improvements were devised for the fractioning of this precipitate. It was found that if the phosphotungstic precipitate were rubbed up with acetone a large part of it went into solution and that the vitamin remained in the smaller insoluble fraction. It was also found that baryta appeared to have a destructive influence on the vitamin, and neutral lead acetate was substituted to break down the precipitate.

The substances isolated by Funk were shown later by Barger (1914) and by Drummond and Funk (1914) to be nitrogenous bases incompletely freed from contamination with the antineuritic factor.

The precipitation of the active substance by silver nitrate and baryta was, however, confirmed by Cooper (1913), by Vedder and Williams (1913), Myers and Voegtlin (1920), Tsukiye (1922), and others.

(2) Suzuki's "Oryzanin."—In 1912 Suzuki, Shimamura, and Odake claimed to have isolated the antineuritic factor from rice in the form of a crystalline picrate. This "oryzanin" is curative in a dosage of 3 to 10 cgrms. Further attempts have been made by Suzuki (1926) to determine its chemical composition but have not proved entirely successful.

(3) Edie, Evans, Moore, Simpson, and Webster's "Torulin."—The above workers in 1912 precipitated a crystalline basic substance of the pyrimidine group from the active fraction of yeast. This they termed "torulin."

(4) Abderhalden and Schaumann's "Eutonin" and "Nutramine" (1918).—These substances have not been generally accepted as concentrated preparations of vitamin B₁.

(5) McCollum's "Dextrin" Method.—In 1918 McCollum devised a method of extracting the active material with hot alcohol, the fatty matter having been first removed by alcohol-free ether. This alcohol extracted residue was then deposited on dextrin by evaporation and further purified by extraction with benzene.

Later experiments by McCollum and Kruse (1926) describe the successful extraction of vitamin B from wheat germ by the addition of a suitable acid (tannin and gallic acids gave the best results) to 95 per cent. alcohol. Two extractions, each lasting half an hour with 0.25 per cent. of these acids in 95 per cent. alcohol, apparently removed the vitamin B completely from wheat germ. After filtration the extract was evaporated and the resulting product tested for its vitamin B content by feeding it to rats that had been kept on a diet deficient in vitamin B.

(6) Osborne and Wakeman's (1919) Preparation.—Osborne and Wakeman's method of concentrating the vitamins from yeast by removing the inert material has been a starting-point for later workers. Moist washed yeast is extracted once with about two parts of boiling 0.01 per cent. acetic acid, and again with about half this amount. It is then centrifuged and evaporated to 0.4 part. This is made up to 52 per cent. by weight of alcohol and the precipitate separated and washed.

The filtrate and washings are evaporated to about 6 per cent. of the volume of the original concentrated extract and made up to 79 per cent. Further precipitations and digestions by alcohol followed by drying produce a friable mass which corresponds to 6.2 per cent. of the dry weight of the original yeast. A given weight of this material has been found to be ten times as potent as the same weight of dry yeast.

(7) Seidell's Method.—Seidell's object in his early investigations (1916) was to obtain the vitamin in a stable form and sufficiently concentrated for use in the treatment of diseases due to vitamin deficiency. He at first used brewers' yeast from which the beer had first been removed by hydraulic pressure, autolysed at 37.5° C. for 48 hours. The thick soup-like fluid was filtered through folded filter papers (pressure filtering failed), and the filtrate obtained was

a clear red-brown fluid about 50 per cent. of the weight of unfiltered material. The effective dose for man would be about 200 c.c.

Various plans for concentration such as vacuum distillation, precipitation by alcohol, etc., were tried, but the method selected as most suitable was removal by adsorption. The adsorbing material used was a hydrous aluminium silicate. To a large volume of clear autolysed yeast filtrate the colloidal silicate was added at the rate of 50 grms. per litre. The adsorbing material with the vitamins was, after a series of processes, collected and dried *in vacuo* over sulphuric acid. This dried material was found to be both preventive and curative in the polyneuritis of the pigeon, and the calculated dose for man would be 10 grms. on alternate days or 5 grms. per day.

In 1922 Seidell described an improvement in the preparation of vitamin-activated fuller's earth. Instead of using a yeast autolysate the method of Osborne and Wakeman was followed, the yeast being treated with boiling water acidified with 0.01 per cent. acetic acid. This treatment has the effect of rupturing the yeast cells and setting free the vitamin which can be readily separated from the residual coagulated protein and other material. The coagulated protein was removed and fuller's earth added to the clear aqueous solution. The fuller's earth, which selectively adsorbs the vitamin, was filtered, washed, and dried, and for convenience was now designated as "activated solid." The vitamin was recovered from the activated solid by rapid extraction with saturated barium hydroxide solution and acidifying the clear extract as quickly as possible with sulphuric acid. This precipitates the barium as sulphate, and addition of solid barium carbonate removes the excess of acid. The filtered neutral solution was then evaporated and yielded a crude vitamin extract which protected pigeons from loss in weight when fed exclusively on polished rice in doses of about 10 mgrms. of the dry extract administered on alternate days.

The vitamin extract prepared as above was subjected to fractionation by silver precipitation, and it was found that a small amount of adenine was present and a larger amount of histidine. The characteristic vitamin action always accompanied the histidine fraction, and it appeared possible that either vitamin was a derivative of histidine or a compound of similar chemical properties which accompanied the histidine as an impurity.

It was found that when the silver was removed from the histidine silver precipitate by suspension in dilute hydrochloric acid, the clear solution could be evaporated to dryness on the steam bath without appreciable impairment of its vitamin activity. The residue so obtained was found to protect pigeons from loss in weight on a diet of polished rice when given in doses of less than 1 mgrm. on alternate days for a period of many weeks.

In 1924 Seidell reported further progress. The material obtained by adsorption was further fractionated by means of picric acid.

In 1926 he described his method as a simple one for preparing large quantities of a highly active non-hygroscopic vitamin concentrate from brewers' yeast. The steps consisted in removing the portion of fresh yeast coagulated at about 90°; adsorbing the vitamin in the aqueous yeast solution by English fuller's earth; extracting the activated fuller's earth with dilute NaOH and promptly acidifying the extract with acetic acid; concentrating by vacuum distillation; removing organic non-active products which separate as the concentration of alcohol is gradually increased; converting the sodium acetate to sulphate by adding an equivalent amount of H_2SO_4 and eliminating it by further increase of the alcoholic content of the solution; finally, converting the active viscous residue obtained by distilling the highly concentrated alcoholic solution to a non-hygroscopic dry powder by treating with absolute alcohol. This final dry product protected pigeons on a diet of polished rice in doses of 0.010 gm. given on alternate days.

A further purification has been effected (1929) by benzylation in alkaline solution and

extracting with chloroform. The aqueous solution remaining after extraction, when poured into 10 volumes of acetone, yields a precipitate of salts accompanied by a nitrogenous compound which protects pigeons from loss in weight on a diet of polished rice in doses containing 0.15 mgrm. of N. Judged on the basis of N activity this final product represents a purification of antineuritic material more than one hundred times that of dried brewers' yeast.

(8) **Hofmeister's Attempt.**—Hofmeister (1920) precipitated the 80 per cent. alcoholic extract of rice meal with potassium bismuth iodide. From the precipitate obtained from the acid solution (after removal of choline in neutral solution) a crude hydrochloride of "oridine" was isolated which was active in doses of 5 to 10 mgrms. On further purification a base, C₅H₁₁NO₂, was isolated which was unfortunately found to be inactive.

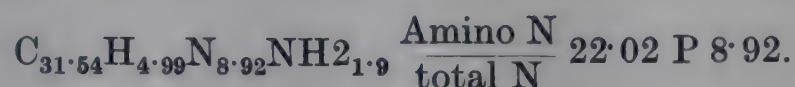
(9) **Levene's Method.**—The original plan of Levene and van der Hoeven (1924) was in a general way similar to that of Osborne and Wakeman, since they aimed to remove gradually the inert material and thus to increase the potency of the active fraction. The purification of Osborne and Wakeman's fraction was accomplished by various reagents of which barium hydroxide, followed by silica gel, were the principal ones employed. The extract at pH 9 of the silica material was very frequently active in quantities of 0.0001 gram and not infrequently in quantities of 0.00005 gram of nitrogen per day. This material, however, still consisted of a mixture of organic and mineral substances even after concentration to such a potency. It was then realised that further progress of the work would depend to a great extent on the reduction of the losses associated with the process of concentration. It was, therefore, attempted to improve every phase in the preparation of the material. The Osborne and Wakeman fraction was purified by shaking with absolute alcohol until it acquired the character of a very fine powder which, on drying under reduced pressure, was no longer hygroscopic. An intermediate precipitation of this material was effected by basic lead acetate before the barium hydroxide solution was added. Selective purification was then accomplished by successive adsorptions with silica gel, and the yield of the substance obtained was between 3 and 5 per cent. of the Osborne-Wakeman fraction. One of the chief impurities now remaining was the salts of phosphoric acid. These were removed from the lead and barium product by precipitating the active material with absolute alcohol from strongly acid solution (pH 2). It was found, however, that the material purified in this manner was not so readily improved by silica adsorption as was the phosphorus-containing material. It seemed therefore that the phosphorus-containing impurity was essential for the selective adsorption by silica. The highest potency of the material obtained by the above process was a daily dosage of 0.0001 gram. In 1926 Levene and van der Hoeven communicated a method of concentration of vitamin B which consisted of two steps: (1) Deamination of the starting material, (2) adsorption of the material on silica gel.

The deamination was carried out by means of barium nitrite and sulphuric acid. An amount of Osborne-Wakeman fraction corresponding to about 6 grms. of nitrogen was taken up in 200 c.c. cold saturated barium nitrite solution, and 10 per cent. sulphuric acid gradually added (butyl alcohol being used to prevent foaming) until the solution was free of barium.

The substance obtained was a yellow powder of an activity of 0.15 mgrm. nitrogen per day, equivalent to 1.4 mgrm. substance. When the observations were extended, however, the material was found ineffective after the first week. The reason for this anomaly was later to be found in the results of research of various workers on the separation of vitamin B into two separate components. Levene and van der Hoeven's material, in the light of these later discoveries, proved to contain the heat-labile, antineuritic factor B₁, in high concentration, but to be deficient in the heat-stable, growth-promoting factor B₂. When 300 mgrms. of heated dried yeast, or 30 mgrms. of heated Osborne-Wakeman fraction, both containing

the heat-stable factor were added to 0.7 mgrm. of Levene's preparation, such a daily dosage sufficed to maintain normal growth.

It was further found that silica gel has the power of adsorbing both factors, but that it adsorbs the heat-unstable fraction preferentially. Thus a unit weight of dried yeast contains 1 part of the heat-stable factor to $7\frac{1}{2}$ parts of the heat-unstable, whereas a unit weight of the material obtained by elution from the silica contains 1 part of the first to 30 of the second. The material obtained was in the form of a slightly yellow powder, completely soluble in water, partly soluble in ethyl and in methyl alcohols, giving negative biuret and Millon tests, and reducing Fehling's solution only after hydrolysis. The substance had the following composition :



Its activity varied, but the best samples were capable of maintaining normal growth in rats in daily doses of 2.2 mgrms. for 18 to 24 days. Continued half-normal growth was maintained with daily doses of 0.7 mgrm.

(a) RELATIVE CONCENTRATION OF VITAMINS B_1 AND B_2 IN LEVENE'S FRACTION.—It was evident that the relative concentration of the factors B_1 and B_2 was not equal. The anti-neuritic factor predominated, for 0.07 mgrm. of the material sufficed to maintain normal growth when 30 mgrms. of heated Osborne-Wakeman fraction were added, while when 0.7 mgrm. of the material were given daily, the amount of treated Osborne-Wakeman fraction remaining unchanged, the growth of the animals exceeded normal growth by 30 per cent.

The relative concentration in yeast could now be determined by the effect of heat. Daily doses of 300 mgrms. of the acetone-dried yeast were required to maintain the normal growth of rats under certain experimental conditions. When the yeast was heated to 135°C . it became ineffective in preventing loss of weight of the white rats. However, when to 300 mgrms. of heated yeast 50 mgrms. of the acetone-dried yeast were added, the growth of the white rats exceeded somewhat the normal. Thus it was assumed that 40 mgrms. of the dried yeast could be regarded as the quantity containing 1 unit of the heat-unstable component and 300 mgrms. as the quantity containing 1 unit of heat-stable component. Hence 300 mgrms. of yeast, containing 1 unit of the heat-stable component, contained 7.5 units of the heat-unstable substance, or the ratio of the first to the second was 1 : 7.5.

The relative concentration of the two factors in Levene's fraction could now be tested by establishing the minimum weight of the material per day required to maintain normal growth when added to the daily dose of the heat-stable material. It was found that 300 mgrms. of heated dried yeast or 30 mgrms. of the heated Osborne-Wakeman fraction contained the daily requirement of the heat-stable factor. It was further found that 0.07 mgrm. of the best samples of Levene's fraction added to 30 mgrms. of heat-stable material sufficed to maintain normal growth. Thus a unit weight of Levene's fraction (approximately 2.2 mgrms.) contained $30 \left(\frac{2.2}{0.07} \right)$ units of the heat-labile substance. Thus the ratio was 1 : 30—a fact which shows that silica gel has a greater power of adsorption of the heat-labile component than for the heat-stable.

(b) PREPARATION OF VITAMIN B_1 FREE FROM VITAMIN B_2 .—Since nitrous acid destroys the activity of the heat-stable factor, the preparation of the antineuritic heat-unstable factor was accomplished by deaminising the starting material with nitrous acid. If the deamination were carried out rigorously, the material did not contain any detectable quantities of the heat-stable material. The best samples obtained in this manner were capable of maintaining normal growth in daily doses of 0.07 to 0.1 mgrm.

(10) Jansen and Donath's Rice Product.—In 1927 Jansen and Donath claimed to have isolated the antineuritic factor from rice polishings in the form of a crystalline hydrochloride.

The formula is given as $C_8H_{10}ON_2HCl$, and it probably contains an amidazol or pyrimidine nucleus. Their method depended partly on the fact that the B₁ factor in rice was found soluble in alcohol to which 0.25 per cent. sulphuric acid was added. The extract was brought up to pH 4 and then treated with acid clay, which absorbed the active material. Further purification with barium hydroxide and silver nitrate yielded a substance which Eijkman (1927) in fact found potent in preventing polyneuritis in chickens in the amount of 2 to 4 mgrms. to 1 kg. of polished rice. Thus the chickens kept in good health on a dosage of 0.1 to 0.2 mgrm. per day. For the rice bird or "bondol" (*Munia maja*) the activity was found to be 0.008 mgrm. per day. In a recent paper, Jansen (1929) describes further improvements in the method of fractionation, as does also Mukherji (1929). The latter extracts the rice polishings with water containing 0.25 per cent. of sulphuric acid instead of with acid alcohol. The growth of moulds is prevented by addition of thymol. Although the yield of active residue obtained is rather smaller using this method, nevertheless the considerable expense involved in using large volumes of alcohol is avoided. In the final purification of the vitamin, Mukherji repeats the platinum purification followed by crystallisation from water instead of the alcohol-acetone fractionation employed by the earlier workers. The crystals obtained were identical in shape with those obtained by Jansen and Donath. The absorption spectrum of the vitamin solution shows marked absorption in the region 390–320 $\mu\mu$.

(11) **Tsukiye's Method (1922).**—Tsukiye has treated an alcoholic extract of yeast with lead acetate, absorbed on charcoal with pH controlled, and precipitated with phosphotungstic acid. The precipitate, containing ammoniacal salts, hypoxanthin, xanthin, phytin, and cholin, is further treated with baryta, ammoniacal silver nitrate, and picrolonic acid, and the resulting substance (still containing impurities) is active in dosage of 0.045 mgrm. for the pigeon, 0.4 mgrm. for the rat.

(12) **Kinnersley and Peters' "Torulin."**—In their preliminary method (1925) Kinnersley and Peters were able to obtain from bakers' yeast, by adsorption with norite, an anti-neuritic substance which was active in doses of 0.5 to 1 mgrm. per day. In later experiments (1928) they have greatly refined their methods, removing the yeast gum by baryta in presence of lead, and adsorbing upon charcoal at pH 7.

Eddy (1928) summarizes Peters' present method as follows :

1. Extract partly analysed bakers' yeast with boiling water. Filter.
2. Treat filtrates with 25 per cent. neutral lead acetate. Filter.
3. Treat with baryta to remove gum. Filter and make acid to Congo red with sulphuric.
4. Remove baryta with sulphuric acid. Filter. Make solution pH 2.5.
5. Add mercuric sulphate in sulphuric acid. Filter.
6. Make filtrate pH 7. Add dry purified norite. Filter after 10 minutes' stirring.
7. Re-treat filtrates with additional norite to complete removal of torulin at pH 7.
8. Extract torulin from norite with N/10 HCl on hot-water bath.
9. Purify torulin by alcoholic fractionation method after the manner of Osborne-Wakeman.

The preparations of Kinnersley and Peters are by this method concentrated to a dosage of 0.027 mgrm. per day, but they have been unable to make Jansen and Donath's method produce the crystalline substance described by them by applying it to their norite fraction. More recently Kinnersley and Peters (1929) have separated in very small amounts a preparation which gave a curative activity of 0.01 mgrm. per day-dose ; this, it will be noted, is the same curative activity as that of the Dutch workers.

There are certain differences between the behaviour of the torulin preparations and those

described by the Dutch workers. The purer torulin preparations do not keep well in alcoholic solution, do not give intense Pauly reactions, and are not precipitable by mercuric sulphate.

The instability of the torulin preparations in ether-alcohol mixtures has not received a complete explanation, but Peters' hypothesis is that treatment with ether removes a stabilising agent. When the vitamin therefore is thrown out in relatively anhydrous ether-alcohol mixtures, and the precipitated vitamin subsequently extracted with ether, the preparation remaining is highly unstable, and though doses given immediately are active, those given later are not.

Possible explanations of the differences in stability and colour reaction between the yeast and rice preparations are (a) that the vitamin forms part of each, but that it is associated with some substance in the rice preparation which makes it stable, or (b) that the two curative substances are different though closely allied in structure.

The conclusion that rice-polishing preparations are different has also been reached by certain Japanese workers. Kinusaga (1927) found that the antineuritic vitamin from rice polishings was precipitable by mercuric sulphate in sulphuric acid solution, thereby confirming the Dutch workers. They have also reached the conclusion that the diazo reaction is not given so intensely by their purer preparation. Their preparations were not very active. Allowing a substantial margin, it seems that the day-dose was of the order of 0.1 mgrm. for pigeons.

(13) **Williams' Activated Fuller's Earth.**—Williams (1928) has succeeded in accomplishing a fractionation of vitamin B by refinements in the control of the adsorbing power of fuller's earth. His preparation prevents polyneuritis in both rats and pigeons in doses of 5 mgrms. of activated earth per day. It resembles Peters' torulin in lacking the heat-stable factor B₂ for rats, but is not identical in its value for pigeons. Williams has so far found it impossible with his preparation to restore pigeons to normal weight by adding autoclaved yeast. These results raise the question whether the heat-labile factors required by pigeons are one or two, i.e. whether the vitamin B complex of yeast is composed of two heat-labile and one relatively heat-stable factor, one of each, or several of each.

(14) **Guha and Drummond's Wheat-Germ Preparation.**—Guha and Drummond (1929) state that they have obtained preparations from wheat germ curative in doses of 0.0025 to 0.005 mgrm. per day, i.e. considerably more active than the activity of the Dutch crystals. These results suggest that more active curative preparations can be obtained than the crystalline ones from rice polishings.

In the last stage of concentration involving precipitation with gold chloride, it was found that the precipitate was slightly active and the filtrate inactive. On combining the two, however, a fraction with great activity was obtained. This suggests that the action of vitamin B₁ is due to more than one factor.

XLVI. CHEMICAL PROPERTIES OF VITAMIN B₁.

(A) **SOLUBILITY**—(1) **In Water.**—The solubility of the antineuritic factor was recognised by Eijkman in 1906.

(2) **In Alcohol.**—In *dilute alcohol* either neutral, acid, or alkaline, vitamin B has been found easily soluble by many workers, including Abderhalden and Schauman, Eijkman, Fraser, and Stanton (1915), and Funk.

In *70 per cent. alcohol*, according to Funk, Harrow, and Paton (1923), and Penau and Simonnet (1921), the solubility appears to be at its maximum, and McCollum and Simmonds (1918) were able to extract it by hot alcohol from beans and wheat grain.

In *absolute alcohol*, or alcohol of high concentration, the degree of solubility has been debated. Abderhalden (1920), Drummond (1917), and Osborne and Mendel (1917) failed to extract the antineuritic factor from yeast by this method, but Fraser and Stanton (1915) and

Funk (1911), using rice polishings, and Cooper (1913), using dried muscle, yolk of egg, and lentils, were successful. Kinnersley and Peters (1928) explain their findings that vitamin B is soluble in alcohol of high concentration by the fact that when other substances which are insoluble in alcohol are removed the antineuritic factor becomes soluble.

(3) **In Ether.**—Generally speaking, vitamin B₁ is not readily soluble in ether, but the degree of solubility appears to depend on the material in which it is found. In dry products, such as beans and wheat grain, it is not extracted by ether, but when ether acts upon an alcoholic extract of vitamin B₁ an appreciable amount is dissolved. An ether extract of egg-yolk has also been found by Cooper (1912, 1914) to possess distinct antineuritic potency.

(4) **In Benzene.**—The same conditions appear to hold for the solubility of vitamin B₁ in benzene as in ether. The natural product cannot be extracted by benzene, but the alcoholic extract can. McCollum and Kennedy (1916) have also succeeded in extracting vitamin B₁ by benzene from wheat germ previously extracted with ether to remove the fat.

(5) **In Chloroform.**—According to McCollum and Simmonds (1918) and Vedder (1912), vitamin B₁ is insoluble in chloroform.

(6) **In Acetone.**—Vitamin B₁ has been found by Abderhalden (1920) insoluble in absolute acetone, but Funk, Harrow, and Paton (1923) have reported it soluble in 70 per cent. acetone.

(7) **In Ethyl Acetate.**—According to McCollum and Kennedy (1916), and in *glacial acetic acid* (according to Levine, McCollum, and Simmonds, 1922), vitamin B₁ is soluble.

(8) **In Olive Oil and Oleic Acid.**—It has been found soluble by Myers and Voegtlin (1920).

(B) **DIALYSIS.**—Vitamin B₁ dialyses easily through parchment, a fact which was recognised by Eijkman in 1906, and other workers, including Chamberlain and co-workers (1911, 1912) and Drummond (1917) have found that the separation is complete when the dialysis is sufficiently prolonged. A suggestion made by Zilva and Miura (1921) as to the molecular constitution of vitamin B has been based on the conditions of dialysis. These workers tested the varying degrees of permeability to substances of collodion membranes by soaking them in alcohol of various strengths. They found that those membranes which were permeable to substances such as methylene-blue, neutral red, safranin, etc., were permeable also to vitamin B₁. They regard vitamin B₁, therefore, as having a molecular weight comparable to these substances (about 300 to 500), or as forming a complex of this molecular weight with some other substance.

(C) **ADSORPTION.**—Vitamin B₁ is readily adsorbed from solutions upon the surface of the following substances :

(1) **Animal Charcoal.**—(Chamberlain and co-workers, 1911, 1912 ; Cooper, 1912, 1914.)

(2) **Vegetable Charcoal.**—(Eddy, Heft, Stevenson, and Johnson, 1921.)

(3) **Fuller's Earth.**—Seidell (1916), Williams (1928), and Randoin and Lecoq (1928) have made use of the selective adsorption of vitamin B₁ by fuller's earth to separate it from vitamin B₂ in yeast.

(4) **Colloidal Ferric Hydroxide.**—(Harden and Zilva, 1918.)

(5) **Colloidal Aluminium Silicate.**—Many workers, including Funk (1916), Eddy (1916), Emmett and McKim (1917), and Levene and van der Hoeven have employed this reagent. Levene and van der Hoeven (1924, 1925, 1926) state that pH 4 is most favourable to its adsorption and pH 9 to extraction.

(6) **Colloidal Silica.**—According to Levine, adsorption is at its maximum at pH 5 and extraction at pH 3 or 9.

(7) **Precipitating Reagents.**—Many apparent reactions of precipitation are in reality adsorptions, e.g. basic lead acetate ; barium acetate, barium and lead sulphate, picric acid, bismuth and potassium iodide, phosphotungstic acid, etc.

XLVII. STABILITY OF VITAMIN B₁.

(A) **HEAT.**—The resistance of vitamin B₁ to heat is considerable, but so much less than that of vitamin B₂ that it is sometimes termed the “heat-labile” factor in contradistinction to the “heat-stable” factor B₂.

The effect of heat on vitamin B depends to some extent on the amount of moisture present; moist heat being much more destructive than dry, and vitamin B-containing foods with a high content of water being much more sensitive to heat than dry products.

Grijns (1901) found that 1 to 2 hours' exposure to 120° C. destroyed the anti-beri-beri properties of unmilled rice, Katjangidjo beans, and buffalo meat; and Eijkman (1906) found horse-flesh still potent after heating for 2 hours at 120° C. Holst (1907), however, found some loss of antineuritic potency in beef heated to 110° C. for half an hour, but none in the case of dried peas or unpeeled barley kept at 115° C. for half an hour.

Nitzescu (1923) states that the vitamin B contained in grains is affected by 100° C. and practically destroyed by 120° C. for one hour in a moist atmosphere, while it remains unaffected by 120° C. dry heat.

Sherman and Grose (1923) have studied the destructive action of gradually rising temperatures on concentrated tomato juice. Their results are given as follows:

Heating for 4 hours at:	Percentage Destruction.
100°	20
110°	33
120°	47
130°	55

They believe that the destruction of vitamin B by heat is a process quite different from the coagulation of proteins, and suggest that the vitamin is combined with or absorbed by colloidal matter rather than present in true solution in water.

From the experiments of Chick and Hume (1917) on wheat germ (natural condition 14 per cent. moisture) and yeast extract (containing 65 per cent. water) it would appear that the destruction of vitamin B₁ takes place very slowly at 100° C. but is much more rapid in the neighbourhood of 120° C. It is concluded from these results that in the baking of bread or biscuit, when the interior of the material does not rise above 100° C., there is no serious destruction of vitamin B₁.

Pasteurisation of milk has, according to the report by Dr. J. M. Hamill, issued in 1923, practically no effect on its vitamin B content.

When heat is applied in an open vessel the vitamin B in rice polishings is destroyed by a temperature of 120° C. for 30 minutes, while in a hermetically sealed vessel no destruction is observed by 134° C. for 90 minutes.

Scotti-Foglieni (1925) finds that a volatile substance with some feeble curative action against polyneuritis is given off with the steam arising from vitamin B₁-containing products heated to a temperature of 130° C., and that when this substance is returned to the residue perfect curative action is restored. From this fact he concludes that vitamin B₁ contains two elements differently affected by high temperatures.

(B) **COLD.**—Freezing has, according to the results of A. H. Smith's (1922) investigations, no effect on the vitamin B present in ice-cream, nor, according to Weill, Mouriquand, and Michel (1916), in frozen meat.

(C) **OXIDATION.**—Vitamin B₁ is apparently not susceptible to oxidation by atmospheric oxygen, differing in this respect from vitamins A and C. Extract of yeast was found by Funk and Dubin (1920) undiminished in potency after exposure to oxygen for 7 hours. Even ozone, according to Zilva (1922), was non-injurious to autolysed yeast after 16 hours' contact.

According to the experiments of Sherman and Burton (1926), on the rate of destruction of vitamin B in tomato juice at varying hydrogen-ion concentrations, oxidation does not seem to have played any appreciable part; the destruction reaction, whether by hydrolysis or by intramolecular rearrangement, was catalysed by OH ions. On both sides of neutrality the rate of destruction was a function of the pH. The extent of the destruction of vitamin B in tomato juice (pH 4.28) upon heating for 1 hour and for 4 hours at 100° C. was measured in over 200 quantitative feeding experiments. Of the total initial vitamin B, the percentages destroyed by 1 hour at 100° C. were as follows: at pH 5.2, about 10 per cent.; at pH 7.9, 30 to 40 per cent.; at pH 9.2, 60 to 70 per cent.; at pH 10.9, 90 to 100 per cent. The corresponding percentages destroyed by 4 hours at 100° C. were: at pH 4.28, about 20 per cent.; at pH 5.2, about 31 per cent.; at pH 7.9, about 70 per cent.

(D) **PRESERVATION**—(1) **Time**.—In dried foodstuffs, according to Jansen (1923) and Findlay (1923), vitamin B₁ withstands destruction for long periods.

Dried beans have been recovered in China and elsewhere from storage of many years and found to contain practically all their original vitamin B.

(2) **Canning and Tinning**.—In these processes of preserving foodstuffs the chief factor to be taken into consideration is the temperature to which they are subjected. Tinned beef, for example, tested by Chick and Hume (1919), did not produce cure in pigeons in amounts equivalent to 106 and 112 grms. dry weight, whereas the curative dose of raw meat was equivalent to 30 grms. dry weight.

Bidault and Couturier (1920, 1921) have also found the action of heat in preserved meats an important factor in their vitamin content. Tinned tomatoes on the other hand are rich in vitamin B₁, and condensed and evaporated milks have been investigated for their vitamin B content with varying results, which, in the light of present knowledge of vitamins B₁ and B₂, may have been reached owing to the failure of some observers to differentiate between them.

The experiments of Daniels and Brooks (1927), for instance, on evaporated milk, when carried out on rats, appeared to show complete destruction, while on pigeons the antineuritic deficiency was only partial.

(3) **Desiccation**.—Vitamin B₁ withstands desiccation well, as might be expected from the fact that its chief sources are dried foodstuffs.

Dried milks, obtained by modern processes of manufacture, where the temperature does not exceed 100° C., probably contain all the antineuritic vitamin originally present. Skim-milk powder was found by Johnson and Hooper (1921) to be of rather feeble antineuritic potency, 30 per cent. of the daily ration being necessary to ensure full protection against polyneuritis in pigeons. These results, however, obviously depend upon the original vitamin B content of the skim milk.

The experiments of Daniels and Brooks (1927), quoted above, show a partial destruction of the vitamin B₁ in milk by evaporation.

The chemical preservation of Chinese eggs in dry-slaked lime and soda process of manufacturing the article of diet known as "pidan" has been found by Tso (1926) to destroy the whole of their vitamin B activity, though there was no apparent loss of vitamins A or D.

(E) **ALKALIES**.—Vitamin B is destroyed by alkalies, but only at high temperatures and high concentration and long exposure.

Boiling with dilute alkali was found by Daniels and McClurg (1919) to leave intact the antineuritic factor in navy and soy beans.

Osborne and Leavenworth (1921) have shown that complete destruction takes place at temperatures of 90° C. with solutions of caustic soda of 5 per cent. concentration, while Williams and Seidell (1916) state that autolysed yeast preserves its vitamin B₁ content under the action

of 10 per cent. solutions of soda at ordinary temperatures. The antineuritic factor in fuller's earth, under the same conditions, remains unimpaired.

Sherman and Burton (1926) consider that the destructive action of alkalies is exerted by means of internal hydrolysis of vitamin B.

(F) **ACIDS.**—Vitamin B is not destroyed by acids. Hydrolysis by sulphuric acid at 5 to 10 per cent. at 100° C. for several hours not only has no destructive action but has been used by Funk as a method of concentrating vitamin B. Hydrochloric, nitric, and nitrous acid have been tested by McCollum and Simmonds (1918), and by Steenbock (1917), Peters (1924), and Kinnersley and Peters (1925), and have been found to have no destructive action.

(G) **FERMENTATION.**—Extracts of the antineuritic vitamin, prepared by extracting with dilute acetic acid, treating with lead acetate and filtering are stated by Rosedale and Oliveiro (1928) to undergo destructive fermentation, especially if left in a warm room.

(H) **ULTRA-VIOLET RADIATION.**—The results of various workers on the destruction of vitamin B by ultra-violet rays are not in entire agreement. Spinka (1924) and Zilva (1919) found that the vitamin B complex in yeast was unaffected by irradiation.

Later workers, however, including Williams (1924), have stated that irradiation of aqueous yeast extract destroys the antineuritic factor. Chick and Roscoe (1929) also found the antineuritic factor destroyed, though to a less degree than the antipellagic. Their results were in opposition to those of Hogan and Hunter (1928) who found vitamin B carriers inadequate to produce growth after exposure to ultra-violet rays for 10 hours but found them still active as antineuritic agents.

Kennedy and Palmer (1929) have concluded that the antineuritic factor is impaired by irradiation, though to a less degree than the growth factor.

Steenbock *et al.* (1929) investigated the effect of irradiation on the vitamin B content of cereals and found no deleterious effect.

XLVIII. VITAMIN B₁ AND METABOLISM.

The net result of all investigations into the requirements of the body with regard to vitamin B₁ tend to show that it is a structural and metabolic necessity of all living cells. The exact nature of its utilisation is a question beset by difficulties, particularly with regard to the factor of the vitamin B complex chiefly concerned. An instance of these difficulties may be found in the subject of lactation requirement of vitamin B. There seems to be general agreement that the nursing animal requires an increased amount of vitamin B in order to suckle her young successfully, but there is a difference of opinion as to which factor should be supplied in extra amount. Evans and Burr (1928), for example, emphasise the importance of B₁ in this respect, while Sure (1928) thinks that B₂ must be increased as well as B₁.

The French writers, Rathéry and Kourilsky (1928), offer another solution of the problem by introducing the conception that the factor chiefly concerned with metabolic processes is neither the "antineuritic" nor the "antipellagic," but the "nutritive" factor postulated by Randoin and Lecoq (1926). Since this factor occurs, together with the antineuritic factor in brewers' yeast, and is not found with the antipellagic factor in autoclaved yeast, it is impossible to deny that the metabolic processes in which vitamin B₁ has been hitherto believed to be concerned may be more intimately connected with this factor of "nutritive utilisation."

In the following account of the action of vitamin B in metabolism, it will be assumed, pending further proof of the separate existence of the "nutritive factor," that the antineuritic is the factor concerned.

(A) **BASAL METABOLISM.**—That a fall in the basal metabolic rate does occur during vitamin B₁ deficiency has been the experience of most workers, but the question which has

produced most divergence of opinion has been that of the specific relation between this fall and the avitaminosis. Since B vitamin deficiency is invariably accompanied by lack of appetite and consequent inanition from starvation, the difficulty in distinguishing between the fall of metabolism due to starvation from that due to vitamin deficiency is very great. In the experiments of Okado, Sakurai, *et al.* (1927), the interesting fact has emerged that the metabolic rate is normal in uncomplicated beri-beri, but falls when a further deficiency of vitamin B₁ is instituted or when cardiac incompetence, paralysis, and atrophy became marked. At the same time, these experiments showed that a partial avitaminosis, induced by a deficient diet, was accompanied by a distinct fall of metabolism at the onset of the initial symptoms, with a tendency to rise later. Where no symptoms were produced by a slightly deficient diet there was no alteration in the basal metabolism.

Verzar and Vasarhelyi (1924) have attempted to explain the fall in basal metabolism by the simultaneous occurrence of a diminished thyroid secretion in vitamin B deficiency. Extracts of the thyroid from beri-beri pigeons failed to augment the metabolism of muscle to the same extent as extracts of the normal gland. The relation between vitamin B deficiency and the function of the thyroid gland has also been noted by Pighini (1927). His results showed that thyroid pulp from beri-beri pigeons was quite without effect on the metamorphosis of tadpoles, whereas the thyroid pulp of normal pigeons provoked marked acceleration in the metamorphosis, and he concluded that the thyroid gland of pigeons suffering from vitamin B deficiency has more or less lost its katabolic morphogenetic functions.

(1) The **Respiratory quotient** has usually been found to be lowered. Abderhalden and co-workers (1920, 1921, 1922), Caridroit (1922), and others have found it as low as 0.75 to 0.78, and Ramoino (1916) even lower—0.44. Van Berkhout (1926) found a distinct decrease in CO₂ production in pigeons fed on polished rice, but in the early stages this reduction was not constant.

Drummond (1926), however, has explained the deficient output of CO₂ recorded by van Berkhout and other observers as due to the relative lack of movement exhibited by vitamin B-deficient animals.

Other workers, especially Magne and Simonnet (1922), have found no consistent lowering of the respiratory exchange in pigeons suffering from avitaminosis; their results could always be found conformable with those of starvation.

Lawrow and Matzko (1926) have even noted an increase in the oxygen consumption and production of carbon dioxide after prolonged administration of polished rice, whereas these factors remained constant when the diet was normal.

(2) **Dynamic Effect of Amino Acids in B Avitaminosis.**—It is suggested by Inawashiro (1929) that the specific dynamic effect exerted by amino acids on oxygen uptake is lessened in B avitaminosis.

The introduction of 1.0 gram of glycocoll into the gizzard of healthy pigeons brought about an average increase of the oxygen usage of over 20 per cent. at the end of 2 hours. A similar procedure with rice-fed pigeons and with pigeons suffering from B avitaminosis resulted in an average increase of oxygen uptake of only just over 7 per cent.

Inawashiro attributes this diminution of effect to an interference in the avitaminous animal with the oxidation of the oxy- and ketonic acids derived from the amino acid.

(3) **Tissue Oxidation.**—A good deal of evidence has been brought forward to show that when the diet is deficient in vitamin B tissue oxidation is diminished, and for some time the "oxidation theory" was widely accepted. This theory suggested that the tissues in vitamin B deficiency have lost their normal oxidising power, and that this can be restored by the administration of extracts of vitamin B.

(a) **EVIDENCE IN SUPPORT OF THE OXIDATION THEORY.**—Dutcher (1918) explained this

loss of power by concluding that the catalase activity of the organs is diminished. Abderhalden and co-workers (1920–1922) and W. R. Hess (1921–1922) also supported the theory, the latter adducing the explanation that vitamin B consists of some substances necessary for the proper functioning of oxidation enzymes present in the tissues. Hess based his explanation on the fact that sublethal doses of potassium cyanide, which is known to diminish tissue respiration, produce a condition like beri-beri, and under the influence of such dosage deficient animals succumb much more quickly than normal.

Hess (1921) attempted to prove that administration of yeast would restore the tissue oxidation to its normal level with the following somewhat indefinite result :

	PIGEONS.		
	Normal.	Deficient.	Deficient, receiving Yeast.
Kidney	23·2	9·4	12·25
Liver	25·5	7·96	7·5
Brain	6·16	3·81	7·5
Muscle	0·95	0·62	0·6

Dutcher obtained more convincing results, and Abderhalden and Wertheimer (1921) were able to confirm them, but found that other substances, such as glutamic and valerianic acid and tryptophane, had a similar effect.

Kollath (1926) followed up the evidence for diminished tissue respiration by investigating the pathological processes in the reticulo-endothelial system. Vital staining with alkaline-methylene-blue showed a diminished reducing power in beri-beri pigeons. Vital storage, as shown by staining with lithium carmine and trypan blue, was increased in the diseased birds. Kollath suggests that in beri-beri there is a disturbance of the tissue and cell-membrane permeability. This alteration in permeability was shown to be still further increased by histamine. There was also found to be alteration in the oxidation-reduction potential of the tissues.

Roche (1928) found that the feeding of vitamin B to a dog in which the urinary excretion of carbon and the C/N ratio had been increased by a diet deficient in all vitamins cured this metabolic disorder. No other substance fed produced this effect.

In later experiments (1929) Roche compared the nitrogen balance, the urinary nitrogen and ammonia and the urinary C/N ratio in fasting rats and in rats suffering from lack of vitamin B. Six rats were used in each group. The fasting animals showed a considerable loss of nitrogen, while the B-deficient rats showed a positive nitrogen balance until 10 days before death, and, during the last 10 days preceding death, their nitrogen balance was only slightly negative. Determinations of the urinary total nitrogen and ammonia nitrogen in the B-deficient rats during the last 5 days before death showed a gradual fall in the former corresponding to the decreased food intake, while the latter remained fairly constant. On the other hand, death from starvation was immediately preceded by a rise in urinary total nitrogen and ammonia nitrogen. The urinary C/N ratio was found to decrease in starved animals and to increase in animals dying from lack of vitamin B. Roche concluded that while partial starvation may enter in as a factor in the death of vitamin B-deficient animals, it cannot be assumed that the death of such animals is primarily due to starvation.

Additional support of the oxidation theory has been offered by the researches of several workers on the glutathion content of tissues.

Glutathion is a dipeptid consisting of a molecule of cystin combined with a molecule of

glutamic acid. The formula is a complicated one, the importance being, it appears, the possession of an SH group and the power of reduction of sulphur derivatives, cystin, thioglycolic and thiolactic acids. The chemical reaction which it undergoes is a to-and-fro one, reduction and oxidation, and it is a typical example of a body which can act in both these ways. The study of its action is indicated in pathological conditions accompanied by disturbance of cellular function.

Randoin and Fabre (1927) compared the glutathion content of the tissues of undernourished pigeons (*i.e.* on a diet inadequate in amount though containing vitamin B) with those on a vitamin B-deficient diet. In the undernourished group the glutathion content was diminished in all the tissues examined, but the diminution was greatest in skeletal muscles. In the group on the vitamin B-deficient diet there was no change during the first period up to the twelfth day. During the second period, characterised by lowering of temperature and beginning paralysis, the glutathion was diminished in skeletal muscles. During the third period (temperature very low, and paralysis well developed) the heart and blood showed some diminution.

In de Mattei's experiments (1928), symptoms of avitaminosis were produced by feeding pigeons on polished rice and husked sunflower seeds, and cure could be easily obtained by addition of yeast. On this diet, in 10 to 15 days, when digestive disturbances and paralytic symptoms appear and the temperature begins to fall, the glutathion figure is lowered slightly, and this diminution becomes very marked as the symptoms progress, except in the liver, where the glutathion tends to increase. When the typical picture of beri-beri was established the glutathion content of the various organs was estimated by Tunncliffe's (1925) method. The results showed a diminution of the amount of glutathion in the liver, blood, kidneys, heart muscles, pectorals, and limb muscles, but since the diminution rarely exceeded the limits of normal variations it was not sufficient to be definitely characteristic of B₁ avitaminosis.

(b) EVIDENCE OPPOSED TO THE OXIDATION THEORY.—Later researches, founded chiefly on the work of Drummond and Marrian (1926), have led to the abandonment of the oxidation theory.

Drummond explains the contradictory results of other workers by his experience of the behaviour of normal tissues under different conditions of temperature. Rats which had been kept on a B-deficient diet for 35 days and starved for 24 to 36 hours had a low body temperature. If this temperature was raised by immersion in a bath, they showed an increase, not a decrease, of the metabolic rate. Similar results were obtained in testing the oxygen consumption of starving rats. At normal temperatures the oxygen consumption was normal, but when the temperatures were low the consumption rose with immersion in a warm bath. Drummond's own results in testing the oxygen intake of tissues by their capacity for decolorising methylene-blue showed no depression in the case of a beri-beri animal, and no alteration in the reducing power of such tissues after the administration of yeast extract. Drummond believes, therefore, that the difference in metabolic rate observed in animals on a B-deficient diet arises not from the specific avitaminosis but from the fall of temperature produced by the starvation associated with the deficiency.

With regard to glutathion metabolism, Yaoi (1928) found no diminution in the glutathion content of muscle taken from pigeons fed on vitamin B-deficient diet, though he found such muscle to have a weaker reducing action on methylene-blue than normal pigeon muscle.

(B) CARBOHYDRATE METABOLISM.—Since Funk (1914) first observed that the effects of B vitamin deficiency were accelerated in pigeons by increasing the amount of carbohydrate in the food, it has been frequently suggested that vitamin B is specifically concerned with carbohydrate metabolism. Abderhalden and Lampé (1913) found that an aggravation of symptoms corresponding to that which occurred with the addition of carbohydrate did

not take place when the protein intake was increased, and the experiments of Braddon, Funk, and von Schönborn (1914), with a further finding of an increase in the blood sugar of polyneuritic pigeons, tended to support this view.

Walsh (1918) also thought that carbohydrate intake must be a direct factor in the immediate production of symptoms of avitaminosis. He suggested that when the vitamin is lacking, the carbohydrate hydrolysis is abnormal, and that the resulting end-products or by-products exert a toxic action.

Eijkman's researches (1916) on the relation between fowl polyneuritis and complete starvation led him, however, to believe that carbohydrates were no more important than protein in bringing about the utilisation of the antineuritic vitamin, and Vedder's experiments (1918) confirmed those of Eijkman. Vedder (1918) stated definitely that the antineuritic vitamin was not concerned in carbohydrate metabolism, and that in arranging a diet to prevent the onset of beri-beri it is sufficient to ensure the consumption of the amount of antineuritic foodstuffs (beans, rice polishings, barley, etc.) that experience has shown will prevent the appearance of the disease, without any reference to the quantity of carbohydrate consumed.

The investigations of Plimmer and Rosedale (1926) upon the vitamin B requirements of growing chicks have shown that the amount of vitamin necessary to ensure normal growth must be varied according to the amount and kind of cereal in the diet. When oatmeal was given 1.5 per cent. of the yeast extract, marmite (the source of vitamin B) was needed to raise the birds to maturity, but with white rice 8.6 per cent. of marmite had to be given.

Randoin (1928) has also made a comparison between the influence of fats and carbohydrates in the diet on vitamin B deficiency. She states that in the absence of vitamin B a diet containing carbohydrate and no fat is not equivalent to a diet containing fat and no carbohydrate, even if the amounts are isodynamic. In rats, as well as in pigeons, a vitamin B-deficient diet containing glucose rapidly brings about polyneuritis, and death occurs in 22 to 53 days. The substitution of fat for the glucose enables the organism to resist the avitaminosis much longer (53 to 134 days). Also, the minimal indispensable dose of vitamin B for the maintenance and growth of rats is very different for the two diets. The one without glucose requires only one-tenth the amount of vitamin B necessary to make the glucose-containing diet adequate.

Gigon (1929) regards the metabolic disturbances in beri-beri as a direct result of carbohydrate excess rather than of simple deficiency of vitamin B₁ in the diet. He states that the disappearance of glycogen in the liver and absence of acidosis are common to both avitaminosis and excess carbohydrate, and that neither occurs with diets containing excess protein or fat.

Plimmer and Rosedale (1927), investigating the balance of various foodstuffs with regard to the body requirement of vitamin B, found that an excess of carbohydrate in the diet, unless the amount of yeast in the diet were proportionately increased, caused pathological symptoms. They suggested, however, that the vitamin B requirement should be expressed by the formula

$$\frac{\text{vitamin B}}{\text{calories}} = \text{constant}$$

rather than the narrower application

$$\frac{\text{vitamin B}}{\text{carbohydrate}} = \text{constant}$$

suggested

by Randoin and Simonnet (1924). With optimum quantities of vitamin B in the diet this constant ranged around 1/40. Randoin and Lecoq (1927) have observed a difference in the requirement of vitamin B according to not only the quantity but the quality of the carbohydrate. A carbohydrate such as glucose needed a larger dose of brewers' yeast to prevent symptoms.

Blood Sugar in Vitamin B Deficiency.—That there is an alteration in the blood sugar during B avitaminosis seems to be an established fact, but whether the most constant alteration is a hypoglycæmia or a hyperglycæmia, or whether, as Drummond (1926) suggests, starvation rather than avitaminosis is the essential causation of the change is not yet decided.

Funk and Schönborn (1914) showed that a hyperglycæmia occurred in birds forcibly fed on rice. The existence of such a hyperglycæmia was not confirmed by the experiments of Eggleton and Gross (1925) on rats, even in the later stages of the disorder. Moreover, the forcible feeding experiments have been criticised by Kon and Drummond (1927) on the ground that often large amounts of a hypertonic solution of the carbohydrate in question must be introduced into the crop. An initial period of hypoglycæmia has been observed by Collazo (1923) and by Palladin and Kudriazewa (1924), while Sure (1929) has observed a marked hypoglycæmia when vitamin B was withheld, ample supplies of vitamin B₂ being given. This progressive hypoglycæmia, as distinct from the ordinary pre-mortal fall in blood sugar, was favourably influenced by vitamin B, thus confirming Suzuki's (1926) finding of hypoglycæmia in cases of infantile beri-beri.

Rose, Stucky, and Mendel (1930) also report an initial hypoglycæmia followed by a moderate hyperglycæmia. In Drummond's experiments (1926) there appeared to be a definite hyperglycæmia in the rat during the greater part of the period. It is kept on a ration deficient in vitamin B. During the final stage of decline, when the body temperature and metabolism are also reduced, there was a marked fall in the blood-sugar level. Similar results were obtained with starving animals. In the animals whose body temperatures had been restored to normal level by warming in a chamber there was no appreciable rise in the blood-sugar level.

Recent experiments by Sure and Smith (1929) seem to show that the hyperglycæmia which does occur in B vitamin deficiency is a rise of the apparent, not the true, sugars, *i.e.* that the disturbance is in the reducing non-sugars. They agree with Drummond that the hypoglycæmia is apparent only in the final stages of avitaminosis and is associated with inanition.

The cause of change in blood-sugar content has been variously interpreted.

(a) THE LACTIC ACID THEORY.—Findlay (1921) observed that the amount of glyoxalase in the liver of pigeons is decreased in absence of vitamin B and increased again when this is administered. Since this enzyme converts methyglyoxal into lactic acid it has been supposed that it is active in carbohydrate metabolism, and that its diminution is accompanied by a lowering of carbohydrate metabolism. Peters (1930) supports this view by his observation that the symptom of opisthotonos in polyneuritic pigeons appears to be due directly to an excess of lactic acid in the brain, and is cured most quickly by injecting concentrated preparations of torulin locally.

Marrian, Drummond, Baker, and Woollard (1927) think that starvation of itself produces it, and attach importance to the fact that the appearance of symptoms is associated with a pre-mortal fall in the blood sugar.

Peters, however, believes that vitamin B is the fraction particularly concerned with carbohydrate metabolism, and especially with the removal of lactic acid in the Meyerhof cycle.

(b) THE PANCREATIC THEORY.—Other workers have concluded that vitamin B acts through the pancreas. Mills and co-workers (1928) found that an acid-alcoholic extract of plants rich in vitamin B reduced the hyperglycæmia and glycosuria of human diabetes but was ineffective in depancreatized dogs. They state that it never produced hypoglycæmia, and that glycogen storage in the liver was increased. Leszczynski (1926) had earlier pointed out a resemblance between vitamin B and insulin in producing a hypoglycæmia—a property possessed also by a series of substances, the so-called glyokinins, which include extracts from onion leaves, tender grass, lettuce, cultures of *B. coli* and *B. subtilis*.

Bierry and Kollmann (1928) have also correlated with the hyperglycæmia found in polyneuritic crises their observation that the pancreas showed enlargement and increase in number of the islets of Langerhans. Their results are not in agreement with those of Hoshi and Ukai

(1926), who, although finding a constant and definite hyperglycæmia in a late period of the deficiency, found no evidence of hypertrophy or increase of the islets of Langerhans.

Racchiusa (1926) has suggested that the hyperglycæmia of polyneuritic pigeons may be due not to a lack of the internal secretion of the pancreas, but to the lowered rate of glycolysis which he has observed. The rate of disappearance of glucose from the blood drawn from fasting pigeons and from those fed on polished rice before the onset of definite symptoms varied little from the normal, but was greatly diminished in the blood from pigeons with fully developed polyneuritis.

(c) **THE STARVATION THEORY.**—The question of the relation between starvation and vitamin B deficiency is really what features of avitaminosis inanition are one-sided, due to lack of a special factor, and what are general to all forms of deprivation of food. As far back as 1911 Chamberlain, Bloombergh, and Kilbourne observed that entire deprivation of food by itself can produce fowl polyneuritis. Eijkman (1916) confirmed this view, adding the further observation that symptoms supervened more readily when water was given copiously.

Drummond and Marrian (1927) showed that in the last stages of avitaminosis the birds lost appetite; if given vitamin B, they immediately recovered it. By forcible feeding it was shown by Braddon and Cooper (1914) and Randoin and Lecoq (1929) that symptoms arose earlier and with much less loss of weight than with natural feeding.

The statements of Kon and Drummond (1927) that carbohydrate metabolism is not controlled by vitamin B are supported by the results of their experiments on starving pigeons. In Kon's experiment (1927) the same amount of food as was consumed voluntarily by the vitamin B-deficient birds was administered to the control pigeons, which also received adequate amounts of vitamin B. No difference could be found in the blood sugar of the two groups. Drummond also found that the blood sugar of a beri-beri pigeon, which was 0.209, as compared with the normal 0.18, remained at 0.209 when yeast extract, enough to supply the B vitamin deficiency, was given as the sole source of food.

(d) **ACETALDEHYDE FORMATION.**—According to Palladin and Utewski (1928) the formation of acetaldehyde in muscle tissue is decreased in both vitamin B₁ deficiency and starvation, and is proportional to the period of each condition. When muscle tissue from either starved or polyneuritic doves was cooked, its acetaldehyde content was nearly normal.

Several workers have attributed the toxic effects produced by administering soluble carbohydrates to B-deficient pigeons to a disturbance of carbohydrate metabolism. Kon (1927) explains the phenomenon to the high osmotic pressure of the solutions administered, and to inability of the deficient birds to empty their crops normally. The absorption of glucose from the alimentary tract has been stated by Pierce and co-workers (1929) to be decreased in vitamin B deprivation. Peters believes that the starvation in vitamin B₁ deficiency is internal, the food being absorbed but not used, and is followed by a secondary loss of appetite. This view receives support from the early experiments of Hopkins (1912) and the observations of Bickel and co-workers (1924) that the nitrogen balance becomes negative in the absence of vitamin B even when the animal is on a diet otherwise adequate.

(C) **PROTEIN METABOLISM.**—The relation between the amount of protein in the diet and the amount of vitamin B required has been investigated by several workers. The early view, upheld by Braddon and Cooper (1914), Funk (1914), Collazo (1924), and Kaczmarek (1925), Hartwell (1921, 1925), and Nelson (1926) was that a high protein diet increases the need for a liberal intake of vitamin B. Later experiments by Sherman and Gloy (1927) seem to disprove this view. Their results show that the protein intake may be changed by feeding either a high or a low protein food as the source of vitamin B without affecting the value of the quantitative test for vitamin B.

The relation of vitamin B to the metabolism of proteins is again complicated by the

difficulty of distinguishing between the action of the various factors in the vitamin B complex. The latest view, quoted by Peters in the *Harben Lectures* (1929), is that the factor concerned with the proper metabolism of proteids is present in yeast extracts containing vitamin B₂. The experiments of Hassan and Drummond (1927) and Hartwell (1928) support the suggestion that B₂ rather than B₁ is the fraction concerned with protein metabolism, and their interrelation will therefore be considered under the heading of vitamin B₂.

(D) **FAT METABOLISM.**—(1) **The absorption of fat** is apparently unaltered in vitamin B deficiency. Tsuji (1922) has found it normal in the dog, and Yoshine (1924) has even suggested an increased absorption, from the fact that the calorific value of the faeces diminishes as the nitrogen balance becomes negative. Cramer (1920), on the other hand, has found a diminished lipid absorption in the rat, but he was dealing with a simultaneous deficiency of vitamins A and B.

(2) **Hyperlipæmia.**—Most workers on this subject, including Asada (1923), Collazo and Bosch (1923), Palladin and Kudriazewa (1924) agree that a hyperlipæmia occurs during vitamin B deficiency. Onohara (1925), however, failed to find it in two vitamin B-deficient dogs. He found the lipocytic index, calculated for the whole body, considerably diminished—0·08 as compared with the normal 0·14.

(3) **“Sparing” Action of Fat on Vitamin B₁.**—Recent investigations (1929) by Evans and Lepkowsky seem to show that the inclusion of large amounts of fat in the diet of rats has a very important effect upon the body's requirements of vitamin B₁. They describe the phenomenon as “the sparing action of fat on the antineuritic vitamin B,” and show that even when there is no vitamin B₁ in the diet, vitamin B₂ being provided in the form of autoclaved yeast, the rat can survive if the diet contains as much as 50 per cent. of fat; if small but still insufficient amounts of vitamin B₁ are given to the rat, the amount of fat which will produce normal growth is less than 50 per cent. The “sparing” action is considered by Evans and Lepkowsky to apply only to vitamin B₁, for when the latter was given, as an alcoholic extract of rice bran, and vitamin B₂ was withheld, the weight curves of the rats are interpreted as showing that the addition of fat had little beneficial effect.

(E) **MINERAL METABOLISM.**—Many observers, particularly Bickel (1924, 1925), have emphasised the similarity between B avitaminosis and mineral deficiency, while others have attempted to correlate the œdema of beri-beri with that due to disturbance of mineral balance, e.g. that signalled by Aubel, Maurice, and Boutiron (1926). Peters suggests in this connection that the puzzling question of œdema (emphasised in the distinction between “wet” and “dry” beri-beri) may find its solution in a factor which co-operates with torulin in regulating the distribution of water in the body.

(1) **Calcium.**—According to the majority of workers the elimination of calcium is unchanged in vitamin B₁ deficiency. Ungar (1927) found the calcium content of the blood serum of pigeons suffering from severe vitamin B deficiency the same as that of normal pigeons, a fact which contradicts the hypothesis that the spasms of avian polyneuritis might be due to parathyroid disturbance.

Palladin and Kudriazewa (1924) have also found the elimination of calcium unchanged in the B-deficient dog, but Miyadère (1922) has found its assimilation diminished in the same animal. The utilisation of calcium is stated by Bogert and Trail (1922) to be improved in man by the addition of yeast to a diet deficient in vitamin B, confirming the earlier results of Schaumann (1910) with pigeons fed on polished rice.

(2) **Phosphorus.**—Most workers, including Palladin and Kudriazewa (1924), Anderson and Kulp (1922), Collazo (1924), and Hirabayashi (1924), have found the metabolism of phosphorus unchanged, but Bickel (1925) and Collazo (1924) have found its elimination increased during the later stages of deficiency, and Bogert and Trail (1922) have found it better utilised when

yeast is added. A low phosphatide content of blood and tissues is stated by Schmitz and Hiraoka (1928) to be a characteristic symptom of B avitaminosis. They believe this deficiency of phosphatides to be brought about by an excess of consumption over synthesis.

(3) **Iron.**—An abnormal distribution of iron in the organs of pigeons suffering from beriberi has been described by Sawanishi (1928). A marked increase of the iron content of the liver and bone, and a decrease in the hæmoglobin content of the blood was restored to its normal distribution by the addition of vitamin B to the diet.

A retarded assimilation of iron has also been recorded by Haramaki (1922) and Yoshine (1922). Suski (1927) carried out experiments to find what influence various Fe compounds may have on the course of the avitaminosis in rice-fed pigeons, and found that Baudisch oxide and iron sulphate, plain and exposed to ultra-violet light, reduced the death-rate and slowed the weight loss.

(F) **CHOLESTEROL METABOLISM.**—A hypercholesterolaemia has been observed by Asada (1923), Hotta (1923, 1924), Kellaway (1921), Nitzescu (1923), and others. According to Stepp (1919) the bile of the vitamin B-deficient dog contains no cholesterol; it would appear therefore that the hypercholesterolaemia is associated with defective elimination of cholesterol by the liver. The cholesterol content of the various organs, and especially of the muscles, is increased, with the exception of the adrenal glands, where it remains normal or slightly decreased.

The following comparison of the cholesterol content of the organs of the normal and B-deficient pigeon has been made by Hotta (1923) and Lawaczek (1923):

	AMOUNT OF CHOLESTEROL PER 100 GRMS.	
	Normal.	B Deficiency.
Blood	0·257	0·436
Pectoral muscle	0·090	0·228
Heart	0·158	0·232
Spleen	0·129	0·245
Liver	0·361	0·372
Adrenal	1·815	1·511
Eyeball	0·128	0·125
Pancreas	0·388	0·441
Brain	1·091	1·163

A connection between the curative effect of turnip juice in avian polyneuritis and its capacity for lowering the cholesterol content of the blood has been pointed out by Horvath (1927). He states that the curative effect cannot be solely due to the richness of turnip in vitamin B, since as a prophylactic measure in doses of 5 c.c. daily it did not always prevent the disease.

(G) **WATER METABOLISM.**—Peters' tests with "torulin" (1930) have revealed a probable connection between vitamin B₁ and the distribution of water in the tissues. Birds fed with salted rice, with the idea of washing out the vitamin by excessive consumption of water, died with massive œdema and dropsy. A bird which received no dose of torulin added to its weight over 100 grms. of water in 2 days preceding death, while one which was dosed with torulin only developed œdema when the torulin was discontinued.

XLIX. VITAMIN B REQUIREMENTS.

(A) **OF ANIMALS.**—There is a marked specific difference in the vitamin B requirements of animals. The experiments of Plimmer (1926) showed that the amount of yeast extract

needed in the diet by different animals was approximately as follows: fowls, 12 per cent.; pigeons, 8 per cent.; rats, 4 per cent.; cats, more than 4 per cent.

Cowgill and Kloty (1927), working on the vitamin B requirements of the pigeon, and following up previous investigations by Cowgill, Smith, and Beard (1925), have concluded that the requirement is in linear relationship to $5/3$ power of its body weight. For the pigeon, rat, and dog, therefore, they state that the vitamin requirement = $KW^{5/3}$ where K is peculiar to the species. Comparisons of the curative activity of Peters' "torulin" preparation for the pigeon and the rat by Reader (1930) show that approximately one pigeon day-dose is needed by the adult rat per day. Since a rat weighing at the time of test 80 to 100 grms. requires the same amount of torulin as a pigeon weighing some 200 grms., the rat requires relatively more than the pigeon. Calculating from these results and basing the B_1 needs upon the rate of metabolism, it may be concluded that some 50 doses a day are required by a full-grown man, or the amount of B_1 in about half an ounce of marmite.

(B) **FOR LACTATION AND THE NURSING YOUNG.**—Sure (1928) has calculated that the lactating mother wastes at least 60 per cent. of the daily vitamin B dosage apportioned to her in the metabolism of transfer to the milk, and thus the large amounts of vitamin B required for lactation are due to the inability of the lactating mother to secrete vitamin B quantitatively in the milk. This point has been stressed by Macy and co-workers (1927) and by Evans and Burr (1928). The latter workers have found that if enough vitamin B is added to the maternal diet the percentage increase in weight from the day of birth to the twenty-first day is equal to that obtained on natural foods (570 per cent.). When yeast is the accessory source of vitamin B, about five times the usual intake is required during the latter period of lactation.

Steenbock, Kent, and Gross (1918), in their early work on barley, had also found an increased intake necessary during the rearing of the young when barley was given as the source of vitamin B.

Evans and Burr's (1928) experiments with tiki-tiki (a dilute alcoholic extract of rice polishings), a substance practically devoid of the growth-promoting factor of vitamin B, indicate that the greatly increased requirement of vitamin B during lactation is due to a temporary lack of the antineuritic and not of the growth-promoting factor. Sure has compared in actual figures the requirements of vitamin B for lactation and optimum growth, and has found that three times as much is required for lactation. This observation confirms the earlier work of Guest, Nelson, *et al.* (1926), who stated that the amount of vitamin B necessary for normal lactation is much greater than that required for normal growth and reproduction. At the same time these workers observed that the amount required for reproduction is much greater than that required for normal growth.

By this method Sure was able to demonstrate that yeasts from various sources vary considerably in their biological value as a source of vitamin B for lactation. A concentrated preparation from yeast, prepared by releasing yeast vitamin B adsorbed on fuller's earth with NaOH, is three times as potent for normal lactation as Harris yeast. It was apparent that yeast administration direct to the young was more efficient than to the mother; the rapid gain in weight of the mothers which sometimes followed the addition of yeast, suggested that they stored the vitamin B in their tissues rather than excreted it in the milk. A judicious distribution between the mother and the nursing young was found to have a good influence on infantile mortality. Whereas 500 mgrms. of a yeast preparation was the minimal requirement of the mother for her litter to remain healthy, it was found that 30 to 40 mgrms. to the mother and 25 mgrms. to each of the young, given direct, was ample. Sure states that nursing young respond remarkably to vitamin B administration, and at the point of failure (with posterior paralysis, muscle tremors, and convulsions) can be saved by

administering concentrated preparations of vitamin B in aqueous solutions with a medicine dropper.

In the case of the cow the vitamin B requirement for lactation is unlike that of any other species of animal. The studies of Macy and co-workers (1927) have indicated that human milk is often decidedly inferior to cows' milk in its content of vitamin B. At the same time the investigations of Bechdel, Honeywell, Dutcher, and Knutsen (1928) had shown that a calf will grow normally to maturity and produce normal offspring on a ration that carries an insufficient amount of the vitamin B complex to support growth and well-being in rats. Subsequently, it was ascertained that vitamin B in milk is not dependent on the presence of this vitamin in the ration of the cow. Cattle differ from the human species in having a rumen wherein foods are subject to vigorous bacterial changes. According to the new observations, in cows on a ration highly deficient in vitamin B, alcoholic extracts of the fermented rumen contents were proved potent in the vitamin B complex through rat-feeding trials. The results of this study warrant the conclusion that the vitamin B complex was produced in the rumen of the experimental cow by bacterial fermentation. This outcome offers a satisfactory explanation for the superiority of the secretion of the bovine mammary gland over the human. Whether the principle of judicious distribution of vitamin B between the mother and the young holds as definitely for human beings as for animals is not entirely established. West (1929) reports that by giving nursing women a diet including raw and cooked fruit, milk, and a tablespoonful of wheat germ daily he has been able to re-establish breast feeding when it was deficient and to improve further the nutrition of the infant by incorporating $1\frac{1}{2}$ teaspoonfuls of dried yeast.

L. EXCRETION OF VITAMIN B₁.

(A) **IN THE FÆCES.**—That the antineuritic vitamin is present in fæces was shown by Cooper (1914). He cured polyneuritic pigeons by administering an alcoholic extract of fæces. Since the discovery of the phenomenon of refection by Fredericia (1925), it has been recognised that the fæces of refected rats are capable of bringing about spontaneous cure in those fed on a vitamin-deficient diet. Fredericia attributes this effect to the presence in the intestinal canal of some virus which produces a substance necessary to correct the deficiency in the diet. It cannot, therefore, be said that refection is a true example of the excretion of vitamin B any more than is the production of vitamin B in the milk of cows on B-deficient diet by bacterial action on the contents of the rumen.

(B) **IN THE URINE.**—The anti-polyneuritic action of urine has been shown by several workers, but the question has been raised as to whether this action is due to the presence of the antineuritic vitamin or to the curative effect of purin bases and other substances. Funk (1912) found this curative action of purin bases so marked that he was led to believe that vitamin B was itself a substance of that class. Sodium chloride and potassium chloride were also found by Eijkman and co-workers (1922) to exert a favourable effect, and distilled water, injected subcutaneously, by Theiler (1915).

In 1918, however, Muckenfuss claimed to have separated vitamin B₁ from human urine, ox-bile, and saliva, by shaking with fuller's earth, and in 1919 Gaglio stated that it was present in the concentrated urine of fasting rabbits, though in smaller quantity than in that of normal rabbits. These experiments have been more or less confirmed by van der Walle (1922) having due regard to the fact that some of the curative action may be due to the presence of substances other than vitamin B.

He concluded that the urine of men or dogs contains a substance which has a strong curative action upon pigeons suffering from polyneuritis. This substance is shown to possess properties similar to those of vitamin B, for it is destroyed by heat, and is adsorbed by

animal charcoal; moreover, it is absent from the urine of dogs fed on a diet free from vitamin B.

LI. PHYSIOLOGICAL ASPECTS OF VITAMIN B₁ DEFICIENCY : BERI-BERI.

The term "antineuritic," applied to the B₁ factor of the vitamin B complex, serves to cover its physiological action in regard to both birds and mammals. The close resemblance between avian polyneuritis and human beri-beri, first pointed out by Eijkman in 1897, has been of great value in establishing the connection of vitamin B₁ with both these conditions, but it has remained for McCarrison (1928) to demonstrate that a condition of beri-beri columbarum, with all the pathological characters of human beri-beri, can be produced in pigeons by means of diets similar to those used by human sufferers from the disease, and that it can be prevented by the same means as those which prevent beri-beri hominum.

At the moment of writing theories are still being advanced which conflict with the more or less universally recognised one of vitamin B deficiency as the true cause of beri-beri. The contention of Matsumura (1929) that a specific beri-beri bacillus is the principal etiologic factor, with deficient diet as a secondary cause, and the theory of Shiroki (1929) that the true nature of beri-beri is an "inanition" of the sympathetic nervous system due not only to the deficiency of vitamin B, but to the complex of various influences on the vegetative nervous system, are examples of this lack of unanimity.

In the last few years McCarrison has repeatedly insisted upon the existence of two types of rice disease in pigeons, called by him "polyneuritis columbarum" and "beri-beri columbarum." The symptoms upon which he relies for his diagnosis of the latter are: (1) enlargement and degeneration of the heart; (2) the presence of œdema or serous effusions, or both; (3) a degeneration of the muscles; (4) chronic passive congestion of the abdominal viscera; and (5) congestion and ecchymoses of the upper intestinal tract. It seems probable that McCarrison's statement, that the clinical and pathological manifestations of beri-beri will ultimately be traced to the operation of a toxic agent produced in the course of a disordered metabolism determined by too little vitamin B in the food, will prove the true explanation. It is interesting to note that this modern view is practically identical with that of Eijkman, quoted by Walshe in 1920.

Beri-beri as a definite clinical entity has been recognised particularly in Eastern countries for many centuries. Findlay (1917) states that it is probably mentioned in writings of as early a date as 2697 B.C., and that it is accurately described in Chinese documents of the second century. An epidemic occurring in a Roman Army in Arabia in 24 B.C. is described by Strabo, writing in A.D. 2.

(A) **GEOGRAPHICAL DISTRIBUTION OF BERI-BERI.**—The endemic occurrence of beri-beri is most marked in a zone of Asia, which includes Japan, the Malay Archipelago, Java, Sumatra, Borneo, New Guinea, and Eastern China; another centre includes the Philippine Islands and Brazil. It has also been observed in Africa (Congo, Madagascar, etc.), in Europe, chiefly in prisons and asylums, and also among the fishermen on the coast of North America.

(B) **ETIOLOGY.**—Even though we hold the vitamin-starvation theory, the ultimate cause of beri-beri may yet prove to be a poison produced by a disordered metabolism arising out of vitamin deprivation.

Various theories have been put forward to explain the origin of beri-beri. They may be classified as follows:

(1) **Diathetic.**—Very early in the study of beri-beri it was recognised that its symptoms must be due to some deleterious, if not actually poisonous, substance present in the circulation. With this in view it was thought to be allied to rheumatism, to scurvy, or to be a form of malaria. Wernich examined the blood in many of his patients, and he concluded that the

disease was similar to pernicious anæmia. This theory, he maintained, would explain the cardiac failure, the dropsy, and the nervous and other symptoms. But it was found that the changes he described in the blood were by no means common to all cases of beri-beri, and that, indeed, many of these changes did not exist in reality, but only in his preparations, the result of his faulty methods of examination.

(2) *Infective*.—In 1885 Simmons wrote in Pepper's *Practice of Medicine*: "Ten years' study and observation of the malady (beri-beri) under a great variety of circumstances and conditions have led me to the definite conclusion that its exciting cause is a specific poison or germ, having many striking resemblances in its mode of production to paludal or marsh miasm, though entirely distinct and separate from it."

Many observers at this period, including Baelz (1882) and Scheube (1884), had reported the presence of micro-organisms in the blood and tissues of patients suffering from beri-beri, but such organisms were of a very wide variety.

Pereira, in 1874, reported the presence in the blood of micro-organisms, which he described as "micrococci in rapid motion." Since he also found these in normal blood he considered them as of no importance.

About 1885 de Lacerda described an organism which he considered to be the cause of beri-beri. This was a bacillus, the same, he says, as he cultivated from rice, and the same as he found in the blood of rats that had died after eating the rice. Some years later, however, in 1887, de Lacerda cultivated cocci from the blood of his beri-beri patients, and by inoculating rabbits with the growth produced the symptoms of that disease. In 1885 Ogata found in the spinal cord of a patient who had died from beri-beri a bacillus something like the anthrax bacillus. Growing in gelatin it had a "tree-like" appearance, and on agar-agar a "yellow-grey-white" colour. On injecting cultures of this bacillus into mice and dogs, he says, there was developed in them the symptoms of beri-beri. He cultivated the organism on a medium of gelatin and grape sugar, and found that the fluid resulting from this growth, on being injected into animals, caused paralysis. He found his bacillus in the intestines of those suffering from beri-beri, and concluded that it was the toxins of these bacilli being absorbed into the circulation that caused the paralytic symptoms. In 1887 van Eecke reported that he had cultivated four different kinds of organisms from the blood of his patients. These were as follows: (1) *Micrococcus albus*, a micrococcus which was immobile, aerobic, and which liquefied gelatin; (2) *Micrococcus tetragenus flavus*, cocci arranged in tetrad forms; (3) *Micrococcus flavus*, also aerobic and liquefying gelatin; and (4) *Bacillus flavus*, short rods in pairs and chains, in active motion, aerobic, and with spore formation. On injecting animals with these growths, three of them—the first, third, and fourth—each produced paralysis. No result was obtained with *Micrococcus tetragenus flavus*.

A more detailed investigation was undertaken in 1893 by Pekelharing and Winkler at Atjeh and at Batavia. At Atjeh, where beri-beri is very prevalent, most of the inhabitants had "cocci and bacteria" in their blood, while at Batavia, where it is rare, no organisms were found. Difficulty was experienced in cultivating the organisms outside the body, and those which did grow showed some variety. Attempts to produce beri-beri by inoculations of the micrococci produced degeneration of the peripheral nerves, but Eijkman (1888) failed to confirm their results, as did also Mendes (1896) and Sternberg (1896). During the next few years, other workers, including Leopold (1892) and Musso and Morelli (1893), continued to claim the isolation of micrococci specific for beri-beri, but their results were questioned by Scheube (1894) and Fiebig (1894), and in 1897 Dr. Spencer summed up the situation by writing: "In spite of the arduous researches of Pekelharing, Winkler, and others, the organism, which is the cause of beri-beri, has not been satisfactorily determined."

In 1897, however, Hunter came forward with what he claimed to be full confirmation of

Pekelharing and Winkler's results. He stated that the staphylococcus of Pekelharing and Winkler, while similar to *Staphylococcus pyogenes albus* in morphological appearance, differed from it in pathogenicity, and was specific in the causation of beri-beri.

In a later paper (1898) Hunter stated that he had cultivated the same staphylococcus from rice used by beri-beri patients, and considered therefore that rice taken as food might be the source of infection. He suggested that rice might be only a suitable medium for the growth of the staphylococcus, and that the entrance of infection might be through the air-passages.

From 1897 onwards the question of the etiology of beri-beri was influenced by Eijkman's description of a polyneuritis experimentally produced in birds, but the bacterial theory still continued, and has continued up to the present day to claim its adherents.

In 1899 Gerrard again brought forward a micrococcus as the specific cause; in 1900 Rost described a diplo-bacillus found in rice water, and later (1906) Tsuzuki announced the discovery of the *Micrococcus beri-bericus*.

Manson (1911) originated a theory that the disease was produced by a toxin from a germ whose nidus was outside the body, a saprophyte in the soil or surroundings which produced the pathological effect of a peripheral neuritis.

Hamilton Wright (*circa* 1907) advanced the view that beri-beri was due to a specific organism which was swallowed with the food and produced in the pylorus and duodenum a toxin which, when absorbed into the system, acted upon the peripheral terminations of the nerves, giving rise to the characteristic symptoms of the disease. This investigator dissented strongly from the view that the essential cause of beri-beri was any foodstuff, or deficiency of any constituent of such foodstuff. Careful investigation, however, failed to isolate the specific bacillus. In 1914, McCarrison, in the course of an investigation into the effect of vitamin deficiency on the endocrine glands, noted the presence of certain bacteria in the blood and tissues of pigeons suffering from polyneuritis. The organisms closely resembled *Bacillus suipestifer*, and living cultures of it produced, in 67·8 per cent. of animals tested, symptoms which were clinically indistinguishable from polyneuritis gallinarum.

In 1923 Noel Bernard and Guillermin drew attention to the action of *Bacillus asthenogenes* on foodstuffs and the similarity of symptoms induced in young pigs by its ingestion to those of human beri-beri. Later (1926, 1927) they isolated a bacillus from the blood in 92 out of 212 febrile cases in men showing symptoms suggestive of beri-beri. The toxin of this bacillus, injected into rabbits, produced gross myocardial lesions or, in smaller repeated doses, showed a selective action on nervous tissues producing paralyses. Positive complement fixation tests against this organism were obtained in the majority of cases of beri-beri examined. These workers therefore regarded beri-beri as a true infection with toxæmia, an excess of rice in the diet being the chief predisposing cause, and not as an avitaminosis with a secondary infection.

The Japanese workers, Tomita *et al.* (1926), obtained somewhat similar results with cultures of *S. Saké*, and claimed that a vaccine prepared from *S. Saké* by heating at 60° C. had a very strong healing power for this type of beri-beri. Spore-forming bacilli of the *B. vulgatus* group were demonstrated by Acton and Chopra (1925) and the suggestion made that water-soluble toxins and neuro-toxins were produced by its action. Investigations by Bose (1924) and by Gloster (1928) tended to show that such bacilli should be regarded only as external contaminants, and did not suggest any causal relation to human beri-beri. During 1929 the infection theory has once more come to the front, though not to the entire exclusion of the vitamin hypothesis. Japanese statistics demonstrate that the epidemic tendency of beri-beri varies from year to year just as is the case among epidemic diseases. Matsumura and co-workers (1929) emphasise the fact that beri-beri appears abruptly along coasts and on the banks of great rivers, and may not appear among people living in far remote areas who are

subsisting on the same type of food. They point out similarly that when a case of beri-beri appears on a ship or in an institution, the disease spreads gradually, and persons living in other buildings may remain unaffected though subject to a similar dietary régime. Beri-beri usually occurs most commonly under insanitary conditions, and there appears to be a predisposition to the disease among those who are suffering from overstrain, insufficient exercise, inadequate sleep, and intemperance.

These workers claim to have isolated a specific beri-beri bacillus (of the *B. coli* group) in the faeces in 74 per cent. of patients with beri-beri, while the incidence in non-beri-beri cases was about 1 per cent.—the average percentage of carriers of intestinal pathogenic bacteria in general.

Further studies showed that fowls with beri-beri had developed specific agglutinations of considerable potency. Usually titres of from 200 to 500 were obtained. If the disease was reinduced, after an initial recovery, the titre not infrequently rose to a higher value—1:1000, or even more. Eighty-three per cent. of agglutination tests performed on 30 consecutive patients with beri-beri were found to be positive, whereas only 7.5 per cent. of 40 normal persons examined gave a positive agglutination test.

Kato (1929) has also investigated the characteristics of the bacillus, with confirmatory results.

The experimenters conclude that “The beri-beri bacillus should be the principal etiologic factor in experimental beri-beri. A deficient diet consisting of polished rice seems to be merely a factor that constitutes a disposition to the disease.”

The *Bacillus asthenogenes* of Bernard (see Plate A) has been again incriminated by Cannon (1929). He states that it exists in two forms: aerobic and saprophytic in the presence of vitamin B; and anaerobic and pathogenic in the absence of water-soluble vitamin B.

The toxin is obtained by filtering through a Pasteur-Chamberland filter candle No. 4. A 1 c.c. dose of the filtrate injected into the vein of the ear kills a rabbit averaging 1800 grms. in weight. For research purposes Bernard concentrates this toxin and obtains a product which kills by a constant dose of 1/20 c.c.

The bacillus can be isolated from human blood for anaerobic culture in a medium consisting of bouillon and milk in equal parts. Only when gastric symptoms exist and at irregular periods can the bacillus be found in the peripheral circulation. In cases of paralysis it is absent, just as the Klebs-Loeffler bacillus is absent in diphtherial paralysis. It can always be found in the spleen.

Various serological researches are carried out by taking a culture of 12 hours' growth and centrifuging. The bottom of the centrifuge is diluted with saline to 0.5 per cent. strength. The best culture medium is pork 25 grms. and saline solution (0.5 per cent.) to 100 c.c. The suspension should be sterilised at 120° C.; 15 c.c. of milk is then added, followed by 10 c.c. of a fresh culture (12 hours' growth), and the whole is heated to 37° C.

The fixation of complement and agglutination test of Bernard is performed with the same culture. For agglutination an emulsion of the microbe is made in 0.5 per cent. saline solution, and is then adjusted to pH 6.5 and heated to 45° C. Agglutination occurs after five hours. For certain diagnosis agglutination only above 1 in 150 must be considered. (Other serums 1 in 50 and 1 in 100.)

Wang (1929) states that this organism gives all the essential characteristics of the *B. cohaerens* of Gotherl, and in Cannon's opinion it is the vitamin deficiency which renders the body very liable to be overrun by the rank growth of bacteria.

(3) **Parasitic**—(a) **ANKYLOSTOMA DUODENALE**.—In the early days of beri-beri investigation a certain amount of confusion arose between beri-beri proper and ankylostomiasis. In 1887 Dr. Kynsey drew attention to the frequent presence of the *Ankylostoma duodenale* in

the intestinal canal of patients suffering from so-called beri-beri. He quoted the opinion of Erni, whose observations were made in Java and Sumatra, that these parasites were found in every case of beri-beri, which he regarded as a helminthiasis. In 1895 Sheperd also described the occurrence of hundreds of worms in the duodenum of native crews suffering from beri-beri, attributing the infection to drinking water. It was immediately pointed out by Seal (1895) that the disease described by Sheperd was not true beri-beri but ankylostomiasis, having none of the peripheral neuritis nor heart involvement characteristic of beri-beri. A later investigation was undertaken by Fraser and Stanton (1909) into the relation between beri-beri and the *Ankylostoma duodenale*, and their results indicated that the parasite played no part in the causation of beri-beri.

(b) **MALARIA.**—Glogner (1899) found parasites in the spleen of beri-beri patients and brought forward the theory, soon refuted, that beri-beri was identical with malaria.

(c) **PROTOZOA.**—Peculiar cells found in the urine of beri-beri patients by Hewlett and de Korte (1907) were regarded as protozoa, the possible cause of beri-beri, and the urine as the source of infection. Daniels (1909), a director of the Kuala Lumpur Research Institute, suggested that beri-beri might be due to a protozoon, the parasite being conveyed to man through the agency of infected rice, but no satisfactory proof of the theory was forthcoming.

(d) **COCKROACH.**—The *Blatta orientalis* (cockroach) was supposed by van der Scheer (1909) to be the cause of beri-beri. He suggested that a part of the life cycle of the beri-beri parasite might take place in the body of the blatta species, which eats human faeces.

(4) **Rice Toxicity.**—Since beri-beri was known to occur chiefly among rice-eating people it was natural that rice should be regarded as a possible toxic agent. Spoiled rice particularly was under suspicion. Mukerjie, for instance, wrote in 1913: "My belief is that beri-beri is due to some peculiar fungus growing in the rice kept in damp godowns for a long time." Before this, Braddon (1907, 1911) had drawn attention to the fact that Chinese and Javanese coolies suffered far more from the disease than Tamils or Malays, and that this difference was apparently due to the method of preparing the rice. The Tamils and Malays always submitted it before it was husked to a process of "parboiling," while the Chinese and Javanese took no precautions of this kind and consumed their rice without any previous "curing."

Although evidence was soon forthcoming from various quarters, including that of Gilmore Ellis (1909), confirming Braddon's view, the mechanism by which white rice produced the disease remained obscure. Some, like Bréaudat (1913), Braddon and Eijkman, and later, Megaw (1923), attributed it to diseased rice, others to the presence of a supposed poison in the rice for which an equally hypothetical antidote existed in the pericarp.

McCarrison's investigation (1923) into the connection between rice-eating and beri-beri in India did not disprove Braddon's theory, but McCarrison laid emphasis upon the endemic occurrence of beri-beri and the protective influence of other foods used to supplement the staple diet. He stated that "unknown factors peculiar to, or more potent in, 'endemic areas' of beri-beri appear to possess an important significance in regard to the genesis of this disease." A specific toxin, oryzatoxin, has been supposed by Ter u-Uchi (1929) to be present in an alcoholic extract of polished rice, but its existence has not been confirmed by the experiments of Sahashi and co-workers (1928) or Miura (1929). The addition of an alcoholic extract of polished rice or its petroleum-ether fraction did not accelerate the onset of polyneuritis in pigeons on Simonnet's diet.

As recently as 1928, evidence arose to restore the factor of deterioration to its place in the etiology of beri-beri, though now with the added consideration of vitamin deficiency. Taylor and co-workers showed the disease to have a seasonal incidence, being most prevalent from August to February; it thus occurs chiefly in the wet period, setting in a couple of

months after the onset of the monsoon. This greater prevalence in the wet season was tentatively correlated with the greater deterioration, at that time, of stored rice from damp and moulds. Where rice was used which was freshly pounded at frequent intervals, beri-beri did not occur, whereas in exactly corresponding communities, where long-stored rice was used, beri-beri broke out. It was not definitely stated whether the moulds introduced a toxic factor, or, as seems more likely, simply made such pericarp as remains on the rice more friable, so that it easily crumbled off and was lost, and with it the vitamin which it contained.

This theory is also held by Megaw, who has recently stated (1930) that it is likely that two or more diseases have been called beri-beri, but these have not yet been differentiated with any conclusiveness; probably one of the diseases might be due to a poison formed in rice which had been stored under hot and damp conditions.

Vergheze (1930) believes that the chief factor in the toxicity of polished rice is the injury to the grains caused by machine milling, allowing the grains to become infected by micro-organisms during long storage in a warm, moist atmosphere.

(5) **Dietary**—(a) **DEFICIENCY OF FAT**.—In 1899 Laurent and Brémand reported that they had been able to stop two epidemics of beri-beri by introducing fat pork, lard, fresh vegetables, and fish. They concluded that beri-beri was an infectious disease attacking people reduced in health by a diet lacking in fat. Laurent's theory was regarded at the time as very feasible, in view of the fact that fat was lacking in most of the diets known to have produced beri-beri, *e.g.* in the outbreak in the Richmond Asylum.

(b) **DEFICIENCY OF PROTEIN**.—In 1880 Takaki succeeded in reducing the incidence of beri-beri in warships from 160 to 16 by means of an "improved dietary." He considered the improvement to lie in eliminating an excess of carbohydrates, and making good a deficiency of protein and avoiding an excess of rice, and attributed the reduction of beri-beri to the increase of protein as such. A later theory originated by Nocht (1908) laid emphasis on the fact that it was not a question of defect of the main components of foodstuffs (protein, carbohydrate, and fat), but of some subtle defect of the less known constituents—enzymes, complements, compound proteins, etc.

(6) **Mineral Deficiency**.—(a) The association of beri-beri with the phosphorus content of rice occupied the attention of a group of observers who followed up Braddon's establishment of the connection between the consumption of polished rice and the incidence of beri-beri. The extensive work of this group, beginning with Schaumann (1908) and including Fraser and Stanton, Simpson and Edie, etc. (1911, 1912), and Vedder (1928), is a striking example of how research, with a fixed conclusion held in view, can lead to erroneous deductions. Schaumann's work (1910) on the "protecting" substances, rich in phosphorus (and at the same time in vitamin B₁), which he found powerful curative agents, shows how nearly he arrived at the true conception of the necessary missing element, yet interpreted his results wrongly.

Substance.	Daily Allowance (Gram).	P ₂ O ₅ Content (Gram).	Protein Content (Gram).
Yeast	1.5	0.063	0.55
Wheat bran	5.0	0.055	0.72
Rice meal	1.5	0.057	0.16

Schaumann took as his starting-point the observations of several workers (Fletcher, 1907; Fraser, 1909; and others) that the abolition of polished rice from the dietary of groups of people among whom beri-beri was prevalent had resulted in a great diminution of the incidence of the disease. Turning to the production of experimental polyneuritis in fowls by Eijkman (1897) and Grijns (1908), he noted, as Holst had done in investigating ship beri-beri

and scurvy in 1907, that not only were the symptoms prevented by the addition of rice polishings, but that the preventive power of protective substances such as fresh meat or Katjang-idjo beans was destroyed by heating to 120° C. Neither Eijkman nor Grijns adopted the simpler interpretation of their facts that beri-beri was a disease due to the deficiency of some essential dietetic constituent in the decorticated rice, but suggested that the endosperm contained some poison, the antidote to which occurred in the cortical layers removed by polishing.

Schaumann now observed that diets which produced peripheral neuritis were invariably poor in phosphorus, and that substances (*e.g.* rice meal, Katjang-idjo) which had the power of preventing the development of neuritis were, on the contrary, rich in that substance; *indeed, that the smaller the percentage of a diet in phosphorus the greater was its influence in producing beri-beri in man, or neuritis in fowls.*

Schaumann thought that the active principle containing phosphorus was probably nucleic acid, and Grijns, following up this hypothesis, experimented with the nucleo-proteins of Katjang-idjo; no curative effect was observed with nucleins extracted by alkali, but some slight postponement of death was observed in neuritic birds treated with the "phosphorus-containing extract" obtained with hot water. He suggested that the phosphatic bodies might in some way serve as sources of energy (physical or chemical) in the nervous system, and that "refreshment of this supply might enable the central nervous system to overcome the hindrance of the degenerated nerves."

Examining the pericarp of the rice which was believed to contain the curative substance, Schaumann stated that it was specially rich in organic phosphorus and in fat, but contained no other peculiar substances, so far as could be determined. Since Grijns had shown that the fat was not the important principle, Schaumann, in his existing state of knowledge, found himself left with only the phosphorus compounds. Independently, in 1909, Fraser and Stanton discovered the same lack of phosphorus in foods which produced peripheral neuritis.

They now decided that the phosphorus content of rice could be regarded as an indicator of its safety as an article of diet, and recommended the establishment of a standard, calculated from the amount of phosphorus pentoxide in the various kinds of rice in use.

They suggested a content of 0.4 per cent. P₂O₅ as the limit for the sale of rice in the Malay States. Later workers, especially Edie, Simpson, and co-workers (1911, 1912), confirmed this standard as a beri-beri-preventing one, and even after the connection between beri-beri and the antineuritic vitamin was firmly established, the phosphorus content of food was still regarded as yielding satisfactory information as to the content of vitamin. Thus Voegtlin and Myers in 1918 made a systematic analysis of different corn products with their methods of estimating the quantity of P₂O₅, and in 1928 Vedder and Feliciano attempted to establish a more comprehensive standard than the 0.4 per cent. P₂O₅ originally proposed by Fraser and Stanton. They suggested "any rice having 1.77 per cent. of phosphorus pentoxide plus fat, but not less than 0.4 per cent. phosphorus pentoxide; or any rice having not less than 0.62 per cent. phosphorus pentoxide; or any rice having not less than 0.5 per cent. phosphorus pentoxide and with at least 75 per cent. of the external layers of the grain thus remaining."

In their later researches into the nature of the substance contained in rice meal which prevented polyneuritis, Fraser and Stanton (1910) demonstrated that it was not a phytin body, nor a fat, probably not a protein, nor did it contain phosphorus. They stated also that the substance was soluble in alcohol and decomposed with sodium hydrate, and, following up their work, Funk was able in 1912 to give a truer definition to what now began to be known as "Vitamin." Schaumann, however, for some time remained in disagreement with Funk's view as to its character and composition. In 1914 he stated that the active substance was split off in the process of extraction from some mother substance present in the food, and that this mother substance was combined with phosphorus in some organic compound.

The real antineuritic principles, he believed, were phosphorus-containing substances which might be either organic phosphorus compounds (the mother substances of vitamins) or bodies which influence phosphorus metabolism (these he called "phosphatesen," of the nature of a ferment). His refutation of Funk's argument is ingenious if incorrect.

According to this theory, in the purification of the phosphorus compounds the lightly attached vitamins in some of them (Phosphatides) appear to get split off; in others (nuclein and nucleic acid) the vitamins are more firmly attached, and stronger chemical measures are required to isolate the mother substances, and in the process the vitamins are separated and destroyed; in this way the phosphorus compounds, which in their natural condition have antineuritic properties, lose them in the extracts partially or entirely. The antineuritic substances increase both the appetite and the metabolism of the body, causing a more complete using up of the carbohydrates and proteids of the food, but they do not affect the phosphorus metabolism. As the quantity of vitamin is so small, it cannot be considered as supplying energy or foundations for cell or tissue formation, but rather to act as an intermediary in certain chemical changes or as a catalisator, and as the animal cannot make these for itself they must be supplied in the foodstuffs.

(b) **POTASSIUM DEFICIENCY.**—In 1910 Kilbourne stated that the potassium content of rice meal was of almost equal value to the phosphorus content as an indicator of its safety.

In 1926 Shiuzo stated that a beri-beri-like disease could be produced in rabbits by a potassium deficient diet independent of a vitamin B deficiency. He regarded the potassium deficiency as being produced by an excess of sodium salt in the diet. The symptoms of this potassium deficiency were chronic paralysis, oedema, slight digestive disturbances, increased heart rate, and a slight rise in the blood sugar resembling the symptoms of beri-beri. There was degeneration of the nerves and muscles, hyperplasia of Langerhan's islets in the pancreas, and some medullary hypertrophy in the adrenal gland, but no ventricular dilatation of the heart or stasis was seen.

Shiuzo suggested that just as vitamin D, sunlight, calcium, and phosphorus all have important effects in rickets, so vitamin B, seasonal influence, sodium and potassium, may all be factors in the production of beri-beri.

(7) **Vitamin Deficiency.**—Although the word "vitamine" entered into the question of the etiology of beri-beri only in 1912, when Funk announced that he had isolated an organic base to which he gave this name, Grijns (1896) long before had clearly postulated that it was due to a lack of "some as yet unidentified food component." Funk himself speaks of Grijns as "the first worker to express clearly a conception of beri-beri which holds good to this day."

Grijns (1914–1927) showed that supplements of mineral salts had no beneficial influence in polyneuritis gallinarum; that the origin of the rice was indifferent; that freshly milled rice was as productive of the disease as that which had been milled a long time, and that additions of fat were not helpful. He found that *Phaseolus radiatus*, the Katjang-idjo bean, as an addition to the diet, was an excellent preventive against beri-beri.

With Funk's definite isolation of a substance contained in rice, possessing curative properties, the field was open for numerous other observers. Modifications of diet in accordance with the established view of beri-beri as a disease due to vitamin deficiency were everywhere reported as leading to its eradication. Chamberlain's report (1915) of the results of giving unpolished rice, beans, and mangoes to the Philippine Scouts, and Chamberlain and Vedder's (1911) use of tiki-tiki (an extract of rice polishings), are examples of the application of the early realisation of the "vitamin deficiency" solution of the problem. During the siege of Kut, beri-beri appeared in the first two months, when the troops received a ration of white flour, but disappeared when one-third to one-half of this flour was replaced by barley

flour and coarse milled wheat flour. From this the following deduction was drawn (see Chick and Hume, 1917):

“For the prevention of beri-beri it is in the highest degree desirable that the germ (embryo) and the bran of wheat should not be excluded from the flour destined for manufacture of bread and biscuit for troops on active service. This is the more necessary when the troops are separated from fresh food supplies and the rest of the ration consists largely of tinned foods, seeing that these articles are deficient in *all* vitamins, owing to their previous sterilisation at high temperatures.”

The investigations of Hehir (1919) into the outbreak of beri-beri among the troops in Mesopotamia in 1915 and 1916 were materially aided by the above report. The Mesopotamia outbreak was obviously unconnected with the rice factor, since rice was never an article of diet amongst British troops. It was controlled by making radical changes in the ordinary ration scale, such as using bread of mixed atta and flour, germinating dhal, etc.

The Far Eastern Association of Tropical Medicine on various occasions from 1910 to 1923 passed resolutions urging the governments concerned to take action to discourage the use of polished rice, and in 1926 further legislative and educational measures were suggested by Chun and Teh (1926). They urged that the former should include the provision of under-milled rice for all public institutions, and possibly the subsidising by the government of mills which would turn out rice milled to the right degree; methods of storage should be controlled by government. The educational measures suggested were the carrying out of extensive and persistent propaganda by the medical profession, public health authorities, and the government, by means of pamphlets, exhibits, cinematograph films, newspaper articles, and lectures in the schools. Not only should the importance of eating unpolished rice be insisted on, but the consumption of fresh vegetables and fruits should be encouraged as much as possible.

Similar recommendations were made by Negishi in Japan in 1927 with the additional suggestion that a mixed diet of rice and wheat would correct the deficiency of the proportion of wheat were fairly large (perhaps 50 per cent.). Even in 1929, however, the present knowledge of the etiology of beri-beri has not prevented an outbreak in a well-organised whaling expedition. Dr. Nissen (1929), the medical officer, reports that the dietary was “too rich,” and traces the outbreak to several causes: (a) The bread was made from finely sifted rye flour of poor quality. (b) The sleeping quarters of the crew were damp and dirty. (c) The potatoes were in an unsatisfactory state, having been stripped two or three times of their sprouts and presumably of their B-vitamins.

(C) **CLINICAL ASPECTS OF BERI-BERI.**—The definition of beri-beri given by Vedder in 1913 holds good in practically every respect to-day:

“Beri-beri is an acute or chronic disease, characterised by changes in the nervous system, and particularly by a multiple peripheral neuritis, with an especial tendency to attack the nerves of the limbs, the pneumogastric and phrenics. Ordinarily the clinical picture of a peripheral neuritis is combined in varying degrees with cardiac disturbances, œdema, serous effusion, and gastro-intestinal derangements. Exceptional cases occur in which cardiac dilatation and sudden death are the first symptoms observed. It is a disease resulting from faulty metabolism, usually only seen in those persons who eat rice as a staple article of diet, and is directly caused by the deficiency of certain vitamins in the food.”

Several forms are sometimes described, the “wet,” the “dry,” the “malignant,” and infantile beri-beri, which, though formerly believed to be an entirely different disease, has recently been recognised as due to vitamin B deficiency also.

As pointed out by McCarrison (1919) and Wenckebach (1928), wet beri-beri and dry beri-beri are essentially the same disease. Wenckebach considers that the so-called dry form is

merely a condition of general atrophy and dryness of skin and tissues found in chronic cases, while McCarrison believes the difference to lie in the greater derangement of the adrenal glands in the wet form. An "acute malignant form" of beri-beri is described by Thierfelder-Thillot (1927) in which the clinical picture is said to resemble that of acute adrenal insufficiency. A pre-beri-beri condition has been described by several observers, particularly Hehir (1919) and Grey (1928). Hehir stated that in the Mesopotamia campaign a number of cases of general debility and physical incapacity, with a tendency to œdema, could be prevented from developing into frank beri-beri by administering a suitable ration at once.

Grey considers that this condition is very prevalent in Japan, and that when it is present beri-beri may appear as the result of many causes without any diminution in the quality of the diet—for example, as the sequel to fatigue, heat, or intoxication—and it is conceivable that a too sudden improvement in the quality of the diet might even act harmfully in stimulating the body too quickly to activity. Prophylactic treatment is by far the most important, since the condition is slowly acquired, and difficult to cure. Milk, eggs, and meat are especially recommended, and natural instead of artificial foods.

He believes that the condition arises from a prolonged use of a diet deficient in essential factors, as well as accessory factors, and in particular from one too low in protein and too high in starch, though there is sufficient vitamin B complex to prevent, under ordinary circumstances, the appearance of beri-beri. It is therefore a condition to which the rice-eating peoples are liable whether the rice is polished or not.

(D) **PATHOLOGICAL CHANGES IN BERI-BERI.**—In addition to the changes described below in the nervous system and the heart, and the occurrence of œdema, beri-beri is characterised by chronic passive congestion of the abdominal viscera, especially of the liver, spleen, and kidneys, and by congestion of, and ecchymoses into, the mucosa of the stomach and duodenum. To these is often added enlargement, with increased epinephrine content of the adrenal glands. McCarrison (1928) states: "None of these conditions are alone diagnostic of beri-beri. But the combination of nervous lesions, enlarged and dilated right heart, anasarca, and effusions, congestion of abdominal organs, and the ecchymoses described, is diagnostic of beri-beri. There is no other disease that presents this peculiar combination of pathological findings."

(1) **In the Nervous System.**—Degenerative processes occur in the peripheral nervous system, damaging and even destroying the peripheral motor and sensory, as well as the sympathetic and parasympathetic, elements.

For many years there was no unanimity as to the exact location of the changes in the nervous system. Some of the contradictions probably arose from the confusion existing in the minds of many observers as to whether experimental polyneuritis and beri-beri were one and the same disease. Marshall Findlay, for example, in 1921, stated: "The only change constantly present in human and avian beri-beri, in the central nervous system, is chromatolysis and nuclear degeneration, more marked in man. The administration of vitamin B, in birds, clears up the paralysis and causes a return of the Nissl granules, indicating that paralysis is somehow connected with their disappearance. These granules are regarded as being composed of nucleo-protein, so that the paralysis of beri-beri would seem to be associated in some way with nuclear starvation."

Most workers seem to have agreed that the general involvement of the nervous system is not so extensive as was at first believed, though Vedder, in 1918, stated that peripheral neuritis *per se* cannot be the essential lesion in beri-beri, because degeneration of the nerves occurs before symptoms arise, because advanced degeneration may be present accompanied by no symptoms at all, and because degeneration of the nerves remains long after recovery has occurred.

Messerle (1926) also stated that changes were present in the spinal cord, consisting of

vacuolar degeneration of cells of the anterior cornu, and swelling and "falling out" of the medullary sheaths.

It has been pointed out by Kon and Drummond (1927) that the prompt alleviation of the acute and nervous symptoms of B vitamin deficiency denotes that true nerve degeneration of avitaminotic origin cannot be present. They add, however, that the paralytic form of pigeons' beri-beri, which is not curable or is curable only with difficulty by vitamin administration, may be associated with nerve degeneration. The acute symptoms are avitaminotic in origin; the chronic degenerative changes are due to inanition and can be induced by under-feeding even when the vitamin supply is not diminished.

Lesions in the spinal cord which they consider to bear a close resemblance to those encountered in human combined system disease have been described by Gildea and co-workers (1930). Dogs fed on a diet deficient in antineuritic vitamin showed a diffuse, irregular loss of myelin, chiefly in the white matter of the spinal cord and less marked in the cerebral cortex. In some cases slight lesions of the peripheral nerves were also found.

REGENERATION OF PERIPHERAL NERVES.—Larcher (1927) has shown that the fibres of the peripheral nerves show active regeneration, even when a neuritic condition has been produced by the avitaminosis.

Other observers, such as Honda (1914 and 1917), Sicard and Roger (1917), and Bito (1928), who have made detailed studies of the nervous system in beri-beri, agree that the principal lesion appears to be in the Schwann cells of the nerve sheath. Bito considers that the action of an antineuritic concentrate is to restore the oxidising power of the protein substance filling the Schmidt-Lantermann segments of the peripheral nerves. A careful investigation by Woollard (1927) revealed no changes in the cord, brain, nerve trunks, or sympathetic nervous system, but definite lesions in the peripheral nerves. The intermuscular medullated sensory and motor nerves and their endings when stained with gold, silver, and methylene-blue methods, revealed definite morbid changes. In some cases the nerve endings had disappeared, but more usually they were swollen, and showed loss of finer differentiation. Contrary to the observations of Culley (1927), who found the axonic changes more marked than the myeluric, Woollard found that the myelin suffered more than the axis cylinder, and the changes were more marked near the ending of the nerve, becoming rapidly less as it leaves the muscle.

Changes in the sensory nerves and their endings in the skin have been described by Tsunoda and Kura (1928) as a "curious primary regressive metamorphosis accompanied by swelling and other changes in their microscopical appearance." After the injection of small doses of vitamin B all these changes and their clinical consequences disappear rapidly. The endings of the sensory and motor nerves improve rapidly within five hours after the injection. Twenty-four hours later the histological appearance is practically normal.

Wenckebach regards these changes, which are not inflammatory in character, as according well with his conception of the "water retention cause of the manifestations of beri-beri."

(2) **In the Heart.**—The importance of cardiac symptoms in beri-beri is emphasised by Aalsmeer and Wenckebach (1928, 1929), especially in the differential diagnosis between beri-beri and other forms of polyneuritis.

According to these observers the most important characteristics of the heart in beri-beri are :

(a) **THE COMPLETE ABSENCE OF PATHOLOGICO-ANATOMIC CHANGES IN THE HEART MUSCLE.**—With regard to the histological changes in the heart muscle, however, it should be noted that the findings of Noel Bernard and co-workers (1927) do not agree with those of Aalsmeer and Wenckebach. They found marked œdema and vascular distension, with some fibrosis in the subepicardium and interstitial tissue of the myocardium. The myocardial

fibres were atrophic, with loss of cross striation, fragmentation, and pigmentary degeneration of the sarcoplasm. The nuclei were proliferated, with hypertrophy and variable deformities. Degenerative changes were also found in the intracardiac nerve fibres and ganglia, the sino-auricular and Tawara's nodes, and in the bundle of His. They draw attention to the resemblances between these cardiac lesions and those experimentally produced by toxic infections in pigs and rabbits.

(b) **THE UNCHANGED ELECTRO-CARDIOGRAM.**—According to Wenckebach (1928) the electro-cardiogram never shows any disturbance of stimulus conduction. The ventricular complex is perfectly normal, and the size of the different waves rather exaggerated, the only peculiarity being a certain degree of preponderance of the right ventricle.

(c) **THE INEFFICACY OF THE CLASSIC CARDIAC DRUGS.**—The whole series of heart remedies and diuretics (digitalis, strophanthin, caffeine, theobromine, thyroid, novarsenol, etc.) has no effect on the beri-beri heart. Vitamin B alone is usually an immediate and complete success.

Radioscopic Examination of the Heart.—Typical cases of beri-beri show an increased cardiac area. The measurements given by Bablet, Guérin, Lalung-Bonnaire, and Pons (1927) are transverse diameter of heart, 13–15 cm.; of pedicle, 7–9 cm.; height of heart, ± 8 cm. In the humid phase of beri-beri, the cardiac shadow enlarges; the diaphragm is unchanged. In the paralytic phase, the shadow increases transversely but decreases in height. The paralysed diaphragm ascends so that the lower cardiac margin reaches the anterior end of the sixth (sometimes fifth) costal cartilage. The cardiac apex is displaced upward and laterally; pulsations are masked by hydropericardium. The cardiac shadow is distinctly triangular.

In the cases examined by Keefer and Hsieh (1929) also the X-ray findings were those of cardiac enlargement, principally right-sided, involving the right auricle and ventricle and the region of the superior vena cava. From the beginning of the disease the whole heart, right and left, is enlarged. This enlargement and corresponding failure of the heart increases more or less rapidly, sometimes in a few hours, but chiefly involves the right heart. Keefer and Hsieh state that the size and configuration of the heart changed completely following treatment, and assumed a normal size and shape after a varying length of time. Wenckebach lays great stress on the fact that the right heart failure and right heart widening and hypertrophy is unaccompanied by pulmonary stasis, œdema of the lungs only showing itself in the last moments before death. As regards the cardiac physics of the condition, Wenckebach brings forward the following argument:

If the two ventricles show an increasing and equal failure in systolic contraction, both will be unable to empty themselves completely at each systole, and the residual blood added to the amount arriving for the next systole will soon bring about cardiac failure. Two factors, however, establish a difference between the right and left side of the heart. Firstly, the right side has a weaker musculature, and the tricuspid valves, having no adequate fibrous support, are very liable to become incompetent. Secondly, since the right heart receives all the blood from the periphery, and since the left heart can only receive that blood sent to it by the right heart, it follows that the more engorged and ineffective the right side becomes the safer becomes the left. These contentions, in Wenckebach's opinion, account for the rapidly increasing failure of the right heart in acute beri-beri, with visceral engorgement, while just up to the last few moments before death the lungs are free, the left ventricle poorly filled, and the pulse small and rapid. The following law is formulated: "In equal and increasing feebleness of the whole heart muscle the patient suffers most and dies from the failure of the right side of the heart only."

The heart failure is believed by Wenckebach to be due to a specific "water retention" swelling (which is not true œdema) of the heart muscle fibres rather than a true hypertrophy.

He, together with Aalsmeer (1928) and with Shimazozo and Honda (1917), does not hold the view that the changes in the heart are due to degeneration in the fibres of the vagus.

Peters (1930) describes a condition of the heart which is manifested by a marked irregularity of rhythm—a slow pulse which becomes enormously accelerated upon exertion. This condition was originally described by Schaumann (1910), and has been further investigated by Carter and Drury (1924). They find that it is due to heart block, apparently central in origin, since it is abolished by cutting the vagus nerve. Carter (1930) has found that the administration of marmite cures the condition very slowly, so that marmite contains only traces of the responsible factor. Cod-liver oil effects no improvement, but whole wheat effects a rapid and complete cure. Peters suggests that this factor may run parallel with the Williams and Waterman factor.

(3) **Œdema.**—The œdema of beri-beri varies greatly in amount, but its localisation is characteristic, invading progressively the lower limbs, face, neck, lower abdomen, and upper limbs.

Deep as well as superficial tissues are involved. Of the serous cavities, the pericardial is most constantly and markedly affected. General ascites occurs in some cases. Œdema of the lungs, not confined to the period immediately before death, was described by Matsuoka in 1915. The three chief theories advanced at the moment as to the causation of the œdema are not entirely reconcilable, and further work is necessary to establish conclusively which is the correct one. The theories of Wenckebach and Peters, however, could, it seems, fit into each other without much difficulty. The three theories are :

(a) **ADRENAL HYPERTROPHY.**—McCarrison (1919) believes that the œdema of inanition and of beri-beri is initiated by the increased intracapillary pressure which results from the increased production of adrenalin, acting in association with malnutrition of the tissues.

He states that wet beri-beri differs from dry only in the greater derangement of the adrenal glands. In his investigations œdema was invariably (100 per cent.) associated with great hypertrophy of the adrenal glands, while 85 per cent. of all cases having great hypertrophy of these organs had œdema in some form. The amount of adrenalin, as determined by physiological methods, in such cases has been considerably in excess of that found in cases not presenting this symptom, and greatly in excess of that found in normal adrenals.

(b) **WATER RETENTION.**—Wenckebach considers the œdema of beri-beri essentially an imbibition process of the tissues. He points out that the swelling of the heart fibres is accompanied by a similar swelling in the skeletal muscles, particularly those of the calf, which disappears less than 24 hours after taking vitamin B-containing food.

Mebius (1928) agrees with this theory and amplifies it by the explanation that the avitaminosis in beri-beri causes a general alteration of the colloidal condition of all contractile tissues. He considers that all the symptoms of beri-beri can be explained by this hypothesis—heart failure, muscular weakness, paralysis of the diaphragm, constipation, œdema, ascites, hydrothorax, hydropericardium.

He states that the sensory disturbances may be caused by the œdematous alteration of the skin, involving also the tactile terminal organs of the nerves; the low tendon reflexes are caused more by the impossibility of contraction of the muscles than by actual neuritis, whilst the tenderness of the muscles is not in contradiction with the still intact condition of the sensory nerves of the muscular tissue.

The theory is further supported by Tull (1928) from his findings of changes in the gall-bladder in beri-beri essentially similar to the changes in the heart muscle described by Wenckebach. He describes an intense œdema of an amorphous coagulable material, especially in the serosa, and between the muscularis and serosa.

(c) "ANTI-ŒDEMA" FACTOR.—Peters suggests that an alkali-labile factor (! B₃) is present in marmite which makes effective the anti-œdematous action of torulin, *i.e.* that torulin plus the "anti-œdema" factor is equivalent to the anti-beri-beri vitamin. This hypothesis is reminiscent of the idea of Vedder and Williams (1913) that there were two forms of beri-beri, one cardiac and the other neuritic, each with a specific vitamin. Peters disputes McCarrison's view that typical beri-beri arises from a diminished amount of vitamin B on the grounds that the administration of large amounts of B₁ does not prevent its appearance. Nor does he agree with the conception of Rosedale (1929) that a separate factor is present in the fractionated precipitate from rice polishings, which is at once effective against wet beri-beri, and against symptoms of intestinal stasis. He suggests that McCarrison was unable to find a strict correlation between œdema and beri-beri because the presence of œdema may sometimes be masked by the absence of salt.

(E) **INFANTILE BERI-BERI.**—Infantile beri-beri is a well-defined clinical entity in the Philippines, in Japan, and in many tropical countries where the expectant or nursing mother is obliged to live almost entirely on polished rice. The condition was first described by Hirota in Japan in 1888. The infant mortality in the Philippines was very great at this time, and in 1904 Hirota's diagnosis was adopted for many of the cases in those islands. That this was correct is suggested by Albert, who in 1908 reported the first case with full clinical and pathological details. Beginning in 1915, the diagnosis was made with increasing frequency in the Panama zone. Recently the tendency to regard infantile beri-beri as occurring exclusively in the young of rice-eating people has been modified by the rapidly growing view that it is the underlying cause of much of the infant mortality in countries where the prevalent diet is deficient in vitamin B₁ whether rice be the staple food or not. (This view receives much support from the investigations of Bray (1928) in the Central Pacific, of Hoobler in the Philippines (1928), and more recently of Ohta (1930) in Tokio.)

(1) **Clinical Manifestations.**—The onset of the disease is usually insidious, beginning either with a heart attack, or gastro-intestinal symptoms, vomiting, anorexia, constipation, or diarrhœa. Œdema is a fairly constant symptom, becoming generalised in the later stages, with evidence of effusion into the serous cavities. In Ohta's cases a paralytic type of the disease occurred; paralysis of the limbs being sometimes accompanied by oculomotor paralysis, ptosis, laryngeal dysfunction, retrobulbar neuritis, and meningeal symptoms.

(a) **IN THE NERVOUS SYSTEM.**—In the cases described by Chapman (1921-1926) the knee jerks were absent or sluggish in some cases, normal and active in others. Hoobler (1928) describes a gradual degeneration of the peripheral nerve fibres, leading to a spastic condition of the arms, legs, and neck with, occasionally, symptoms referable to meningeal irritation, such as choreic movements, twitching of the face, slight rigidity of the neck, general spasticity, squinting, and even convulsions. Bray describes a "meningismic" stage in the acute form, with opisthotonos.

(b) **IN THE HEART.**—The heart is hypertrophied and dilated, the wall of the right heart equalling or exceeding in thickness that of the left heart.

Suzuki (1926) states that in 91 per cent. of infants suffering from beri-beri the heart is hypertrophied during the first week. Bray states that the muscle fibres show patches of cloudy degenerative and early fatty changes commencing more especially near the endocardial surface.

(c) **ŒDEMA.**—The œdema in the later stages is generalised, affecting all the organs and tissues of the body in time, and progressing eventually to a well-marked anasarca. The description given by Bray, though attributed by him to the "incapacity of the heart to meet its obligations," accords well with the water-retention theory since advanced by Wenckebach.

(2) **Prophylaxis and Treatment.**—There is evidence that the milk of women suffering from beri-beri contains considerably less vitamin B₁ than that of other women (Suzuki, 1926, Vedder, etc.). The obvious prophylactic method would seem to be the feeding of the mother with a source of B vitamin. Bréaudat and others (1913) showed that rice polishings and mango given to the mothers would bring about a cure, but the rice polishings (tiki-tiki) were disagreeable to take, and breast-feeding had to be discontinued for a time. This is the method, however, suggested by Hoobler and Bray. The latter has reported good results from the administration of an emulsion of the yeast from "toddy" (the sweet sap of coco-nut spathe).

The introduction of a concentrated form of vitamin B into the diet of infants has been found valuable both in prophylaxis and treatment. McLaughlin and Andrews (1910) made up such a concentrate which consisted of an alcoholic extract of rice polishings (tiki-tiki).

Chamberlain and Vedder (1911) found the extract of tiki-tiki curative for polyneuritis gallinarum and recommended its use for infants; 5 c.c. of the extract represented 82 grains of rice polishings, and the dose advised was 5 c.c. of the extract daily, given in 20-drop doses every two hours. Albert (1915) reported remarkably good results from its use, the disease, except in very severe cases, being practically cured at the end of 3 days.

Wells (1921) described a technique for an improved form of this preparation, separating off the active constituents and concentrating the fluid to the consistency of a syrup.

(F) **EXPERIMENTAL POLYNEURITIS IN ANIMALS**—(1) **In Pigeons.**—The discovery was first made by Eijkman in 1897 of the condition which he called polyneuritis gallinarum, and he demonstrated its causal relationship to rice. Its resemblance in many particulars to the disease produced in human beings by eating polished rice, with simultaneous differences in some respects, led to much confusion and discussion as to whether both conditions were manifestations of the same cause. In 1919 McCarrison drew attention to the pathological differences which exist between polyneuritis columbarum and beri-beri hominum. The table shown on page 256, taken from his paper on "The Pathogenesis of Deficiency Disease" (1918–1919) shows these essential differences:

The Japanese workers, Ogata *et al.* (1924) and Nagayo (1923), recorded even more marked differences in the two conditions, concluding that the two diseases were radically different. Ogata, for instance, stated: "Wide differences exist between the nervous systems in the two diseases"; and "Differences are seen in the pathological changes in the suprarenals, the cortex in avitaminosis being hypertrophied, and the medulla and adrenaline content being normal; whereas in beri-beri the medulla is hypertrophied and the adrenaline content increased. While the generative cells become atrophied in avitaminosis, no obvious change occurs in beri-beri." Nagayo laid emphasis on the "anæmia, lymphopenia, and emaciation present in the 'rice disease' and absent in beri-beri."

A very detailed investigation into the symptoms, pathological appearances, and pathogenesis of avian beri-beri was made by Marshall Findlay in 1921, as follows:

The main symptoms were: During incubation, there was usually some diarrhoea, with light-coloured stools. The actual onset was usually gradual. The feathers became ruffled, and the head was kept sunk between the shoulders, while in walking the tibio-tarso-metatarsal joint was held partially flexed, owing to commencing paralysis of the extensor muscles of the leg. Death usually intervened in 2 or 3 days, the bird, as a rule, becoming semi-comatose before it. Symptoms in detail:

1. **Nervous Symptoms.**—Paralysis first appeared in the flexor muscles of the toes and spread upward; wing-droop was rare in pigeons. Head retraction was marked, from paralysis

TABLE CONTRASTING THE POST-MORTEM APPEARANCES IN POLYNEURITIS COLUMBARUM AND HUMAN BERI-BERI.

Organs.	Polyneuritis Columbarum.	Human Beri-beri.
Heart ...	Atrophy : frequent dilatation right heart : sometimes oedema of heart.	Hypertrophy : frequent dilatation right heart. Weight of heart, 13.37 ounces as compared with 9 ounces in controls (Ellis).
Spleen...	Atrophy pronounced ...	Hypertrophy : weight, 9.27 ounces as compared with 6.28 ounces in controls (Ellis).
Pituitary ...	Tendency to enlarge in males only ...	No records.
Adrenals ...	Marked enlargement with increase of epinephrine-content.	Enlarged (Sprawson) ; epinephrine-content increased in acute cases (Ohno).
Thymus ...	Great atrophy ...	No record : enlargement of thymus referred to by Nagayo.
Testicles ...	Great atrophy ...	No records.
Ovary ...	Atrophy marked ...	No records : amenorrhœa reported (Vedder).
Pancreas ...	Atrophy marked : sometimes ecchymoses	No records : pancreatic insufficiency mentioned by Breaudat and Lalung-Bonnaire.
Liver...	Slight atrophy : frequently hyperæmic ...	Enlarged : usually hyperæmic (Scheube, Bentley) ; rarely congested (Ellis).
Stomach ...	Atrophy ...	Congested in 54.4 per cent. of cases (Ellis) ; hyperæmia, ecchymoses, erosions (Miura).
Duodenum ...	Hyperæmia and ecchymoses : atrophy ...	Hyperæmia and ecchymoses (Scheube) ; duodenitis (Wright).
Intestines ...	Atrophy : hyperæmia : ecchymoses ...	Hyperæmia and ecchymoses, 100 per cent. (Scheube) ; catarrh (Miura) ; congestion, ecchymoses (Wernich, Anderson, Simmonds, and others).
Lungs ...	(Edema rare : weight slightly increased ...	(Edema common : hyperæmia and oedema 100 per cent. (Scheube) ; 36 per cent. (Pekelharring and Winkler) ; 62 per cent. (Ellis) ; 80 per cent. (Yamagiwa).
Kidneys ...	Slight atrophy : frequently hyperæmic ...	Enlarged and congested the rule (Yamagiwa, Ellis, Bentley, Scheube, and others).
Thyroid ...	Slight atrophy : congestion very rare ...	No records.
Brain ...	Very slight atrophy : may be hyperæmic or anæmic or normal appearance.	Hyperæmia and oedema (Scheube) ; oedema (Bentley) ; anæmia (Yamagiwa).
Meninges ...	Occasionally hyperæmic : rarely anæmic	Hyperæmia (Yamagiwa).
Cord ...	Rarely slight atrophy : no change in its membranes.	Anæmic (Yamagiwa) ; congested and oedematous (Bentley) ; oedema (Scheube).
Skin ...	No appreciable change, probably thinner	(Edema : purple or green-grey spots : ecchymoses have been reported.
Subcutaneous tissues.	Complete disappearance of fat : oedema rare.	(Edema common : 55 per cent. of cases (Scheube).
Ascites ...	Rare ...	Common : 50 per cent. (Scheube) ; 40 per cent. (Yamagiwa).
Hydrothorax...	Rare ...	Common : 25 per cent. (Scheube) ; 90 per cent. (Yamagiwa).
Hydropneumothorax.	29 per cent. of cases in one series : 13.1 per cent. in another : 75 per cent. in a series of young birds.	Very common : 75 per cent. (Scheube) ; 98 per cent. (Pekelharring and Winkler) ; 64 per cent. (Ellis) ; 53 per cent. (Yamagiwa).
Muscles ...	Great atrophy : rarely oedematous : often pale or mottled.	Pale, atrophic (Bentley) ; atrophy (Baels, Scheube) ; oedematous (Anderson, Wernich).
Vessels ...	Abdominal vessels often congested ...	Usually congested.
Bones ...	Very brittle : bone-marrow reduced ...	No records.

of the anterior neck muscles, emprosthotonos being occasionally noted. At a late stage dysphagia occurred.

2. *Sensory Changes*.—Apparently there was an impairment of the sensations of touch, temperature, and pain in the legs.

3. *Cerebellar Disturbances*.—Occurred in all but 4 pigeons, though only once in a fowl. Characterised by periodic convulsions, induced by any sudden stimulation.

4. *Temperature Changes*.—On a vitamin B-deficient diet the daily temperature, which in healthy birds ranges from 105° to 109° F., taken in the cloaca, gradually falls to 99°, 98°, or even 96° F. A curative dose of vitamin B will cause the temperature to rise again as the muscular paralysis disappears.

5. *Changes in the Cardiac and Respiratory Functions*.—In beri-beric birds there was a progressive slowing of the respiratory rate, symptoms of air hunger frequently appearing late. There was also a marked acceleration of the heart, occasionally a slowing. Both these symptoms could be relieved by a curative dose of vitamin B.

6. *Edema*.—Not noted in the lower extremities of any of the birds.

7. *Changes in Body Weight*.—On an exclusive diet of polished rice both fowls and pigeons lost weight, the fowls in the course of the experiment 25.6 per cent. of their original weight, or 1.2 per cent. per day, the pigeons 29 per cent. of their original weight, or 2.5 per cent. per day. Often there was a rapid loss of weight shortly before death, sometimes preceding the nervous symptoms.

In fowls fed on water only, death occurred on the average in 11.6 days from inanition, but there were no clinical signs of involvement of the nervous system, progressive asthenia being the main feature. Of the pigeons fed on oats, maize, and yeast, 4 died at an average of 27 days, the other 2 being killed on the thirty-fifth day. No paralytic symptoms were noted, though the respiration was slowed and the temperature lowered as in fowls dying from inanition.

Post-mortem, the most striking feature in the beri-beric birds was the extreme atrophy of all the tissues, except in the case of the adrenals, which in both beri-beri and inanition show marked hypertrophy, and the pituitary, which also in both conditions shows little or no change. Appearance of tissues:

I. NERVOUS SYSTEM—(a) *Macroscopically*.—The brain occasionally showed slight congestion of the meninges or of the brain substance, though it sometimes appeared a dead white. Brain substance usually soft and almost diffuent. No increase in cerebrospinal fluid. No petechia. The cord appeared normal.

(b) *Microscopically*—(1) *Nerve Cells*.—Almost complete disappearance of the Nissl granules, which were replaced by a fine granular mass, usually placed close to the origin of the axis cylinder. Chromatolysis a little more marked than in pigeons fatigued by a long flight. Nissl granules reappeared 6 to 9 hours after a curative dose of vitamin B. In birds dying of inanition, there was only slight evidence of chromatolysis.

(2) *Nerve Processes*.—Fibres stained black with Marchi's method occurred irregularly in the cord and brain, without relation to blood vessels or lymphatics. In a few cases no degeneration could be found, though there was typical paralysis.

(3) *Neuroglia*.—No overgrowth.

(4) *Blood Vessels*.—Occasional congestion. Some muscular fragmentation in the smaller arteries, and in places swelling of the endothelium.

(5) *Peripheral Nervous System*.—As a rule there was degeneration in the myelin sheaths of the sciatic fibres; encountered also in 2 cases of chronic inanition in pigeons. Marchi's method showed degeneration in the vagi, as a rule: this was never encountered in cases of inanition.

(6) *Sympathetic System*.—Degenerative changes in the intestinal plexuses, and swelling and vacuolation of the ganglion cells of the adrenal.

II. ENDOCRINE ORGANS—(a) *Adrenals*.—Hypertrophy is somewhat greater in the female, and occurred, though less markedly, in inanition. Macroscopically, they are reddish copper in colour and friable, sometimes considerably congested. Microscopically, the medullary cells are fairly normal, though many of the nuclei are large and vesicular and show loss of chromatin; there is a marked increase of lipoid material in the cortex, and the oxydase reaction of the cortical cells is increased. Following a curative dose of vitamin B, the weight of the adrenals decreases from a decrease in their lipoid content.

(b) *Pituitary*.—Little or no change, either in polyneuritis or inanition.

(c) *Thyroid*.—Slight atrophy in polyneuritis. In inanition the colloid is decreased.

(d) *Parathyroids*.—Appeared normal, except for slight atrophy of the post-branchial body.

(e) *Thymus*.—Completely atrophied and disappeared in polyneuritis, and in inanition, even when vitamin B was present in the diet.

(f) *Spleen*.—Marked atrophy, both in polyneuritis and inanition, amounting to almost two-thirds of the original weight. Appears dark red and very friable, and shows microscopically a loss of lymphoid cells, with congestion and the presence of large amounts of yellow-brown pigment.

III. GENITO-URINARY SYSTEM—(a) *Genital Organs*—(1) *Testicles*.—Show shrinkage of the tubules, which are filled with cellular debris and show impairment of spermatogenesis. Similar changes seen in inanition. (2) *Ovaries*.—Small, with minute vesicles of uniform size, the membrana granulosa being composed of a single layer of epithelial cells.

(b) *Kidneys*.—Relatively little atrophy. Congestion of the intertubular capillaries with cloudy swelling of the cells of the convoluted tubules. No fatty degeneration noted.

IV. CIRCULATORY SYSTEM—(a) *Heart*.—No great hypertrophy. Right heart frequently dilated, especially the auricle. Myocardium soft and pale, frequently oedematous at the auriculo-ventricular junction. Muscle cells showed cloudy swelling and frequently commencing fragmentation. The changes in inanition were very similar. Hydropericardium was encountered in 26 per cent. of fowls and 20 per cent. of pigeons with beri-beri, and in 16 per cent. of fowls and 16 per cent. of pigeons with inanition, and varied from 1 drop to 12 c.c. in fowls and from 1 drop to 4 c.c. in pigeons. It appeared to have no relationship to the size of the adrenals.

(b) *Blood Vessels*.—No atheroma noted in the aorta. Microscopically, the smaller arteries showed swelling of the intima, with degenerative changes in the muscular coat of the media.

V. ALIMENTARY TRACT—(a) *Æsophagus*.—Thinning from atrophy of the muscular coat.

(b) *Crop*.—Changes similar to those in the æsophagus.

(c) *Glandular Stomach*.—Atrophy of the mucosa, and thinning of the wall. Marked congestion. Disappearance of lymphoid tissue. Cessation of active secretion in the large glands and degeneration of the lining epithelium.

(d) *Muscular Stomach*.—Atrophied, mucosa frequently easily detached.

(e) *Intestines*.—In polyneuritis the atrophy of the wall was visible grossly. Duodenum congested and ecchymosed, not noted in inanition. Microscopically, there was atrophy of the mucosa and muscularis. The lymphoid tissue had largely disappeared.

(f) *Liver*.—Dark red and friable. Gall bladder usually empty, in contrast to cases of inanition. Microscopically, there was usually marked congestion and fatty degeneration, and occasionally cloudy swelling. Many nuclei pale, swollen, and vesicular. Mitoses not seen.

(g) *Pancreas*.—Considerable atrophy. Very pale. Acinar cells showed cloudy swelling, and pale, vesicular nuclei. Islets normal.

VI. MUSCULAR AND OSSEOUS SYSTEMS.—Muscles soft, dark, and atrophied, with loss of cross striations. Bones atrophied and light.

McCarrison (1928) points out that it is not possible to distinguish clinically between polyneuritis columbarum and beri-beri columbarum. In both, the polyneuritic symptoms, the head retraction, the "fits," and other features are to all appearances the same.

Birds, however, do not exhibit œdema of the feet and legs, while their feathered state does not readily permit of the recognition during life of subcutaneous œdema; nor is the recognition of effusion into the serous sacs nor the nature of the cardiac derangement practicable in these animals during life. In making a diagnosis of beri-beri in polyneuritic birds reliance has, therefore, to be placed on the various pathological appearances found at post-mortem examination, the diagnosis being based on the correspondence between these appearances and those characteristic of the human disease. In addition to the nervous lesions the following are the features on which the diagnosis of beri-beri columbarum depends: (1) enlargement and degeneration of the heart; (2) the presence of œdema or of serous effusions, or of both; (3) degeneration of the muscles; (4) chronic passive congestion of the abdominal viscera; and (5) congestion and ecchymoses of the upper intestinal tract. When all these pathological features are present in birds which exhibited definite signs of polyneuritis prior to death, the diagnosis of *true beri-beri of the wet variety* can be made. When all are present except œdema or serous effusions the diagnosis of *true beri-beri of the dry variety* is permissible; when the heart is neither appreciably enlarged nor atrophied, but is obviously degenerated, the diagnosis of beri-beri of a type *intermediate* between the fully developed disease and polyneuritis columbarum may be made; when the heart is obviously smaller than normal, although it may be degenerated, the diagnosis of *polyneuritis columbarum* is appropriate.

These observations have been fully confirmed by other workers, including Graham (1927) and Taylor and Thant (1929), and it is now more or less universally accepted that the determining factor in the occurrence of the different varieties is the degree of lack of antineuritic factor in the diet.

The basal factor in the production of beri-beri columbarum is insufficiency, but not complete lack, of the antineuritic fraction of vitamin B. There is an optimum insufficiency of vitamin B—provided by diets of which the vitamin B value is from 20 to 50 per cent. below the minimum required for the maintenance of normal metabolism—at which beri-beri columbarum is most likely to arise.

(2) *In Rats*.—When rats are fed on diets deficient in vitamin B₁, only a small percentage develop polyneuritis, the majority die of inanition only. In 1917, Sundwall confirmed in the rat the findings of earlier workers on pigeons and hens in regard to the production of nervous lesions by simple inanition. Examination of the spinal cord disclosed congestion of the vessels, vacuolation of the cellular elements of the cord suggesting extreme œdema, and certain abnormalities of the anterior horn cells, some of which were shrunken and stained darkly, whilst in others the vesicular appearance of the cytoplasm was presumptive evidence of chromatolysis. Examination of the nervous system in rats fed on diets lacking the antineuritic vitamin has been carried out by various observers. Schaumann (1910) produced paralysis of the hind legs by feeding rats on an exclusive diet of horseflesh which had been sterilised at 120° C. Examination of the nerves showed no typical degeneration of the myelin sheaths, but a foamy appearance which was considered to be evidence of the breaking up of myelin. Hofmeister (1922) fed rats on a diet which was deficient only in vitamin B. In a few animals a spastic condition of the limbs occurred, accompanied in some cases by tremors

and convulsions, but in most there was at first a definite ataxia of the hind limbs followed within a day or two by paralysis. In the nervous system there were no degenerative changes in the peripheral nerves with the exception of slight swelling of the myelin sheaths in three rats and an increase in the nuclei of the sheath of Schwann in two others. In the brain, brain stem, and cerebellum small capillary hæmorrhages were, however, observed, the changes corresponding roughly to the severity of the symptoms observed during life, since hæmorrhage was absent in those rats which had not shown any nervous symptoms. Degenerative chromatolytic changes were present in the nerve cells of the brain. Hofmeister regarded the hæmorrhages as evidence of a hæmorrhagic encephalitis in spite of the absence of inflammatory change.

Woollard's observations (1926) also showed the absence of Marchi degeneration in the cord and nerves of rats on a diet deficient in vitamin B. There was an absence of chromatolysis in the nerve cells of the anterior horn of the cord, but changes were present in the fine inter-muscular medullated motor and sensory nerves: their terminal ramifications were swollen, particularly in the most peripheral parts, which showed loss of fine differentiation, the myelin being irregular in outline.

Woollard, however, noted similar changes, though less pronounced, in rats which had been completely starved, except for water, for from 4 to 7 days, and also in rats which had received marmite as their sole source of nourishment. He suggests that the more pronounced manifestations of experimental beri-beri may be explained by the different duration of the conditions, and that beri-beri occurring in rats may be equal to starvation from inability to assimilate food in the absence of the appropriate vitamin.

Swelling of the nerve endings of pigeons fed on a diet of polished rice has also been observed by Tsunodo and Kura (1928), who state that the swelling disappears after administration of the antineuritic factor. A description of the pathological changes in the nervous system of rats kept on diets deficient in vitamin B₁ is given by Stern and Findlay (1929), together with a comparison of the changes occurring when vitamin B₂ is added. The chief result of the investigation was to show that diets lacking in one or both of the factors making up the vitamin B complex produce results entirely non-specific in character. Absence of the antineuritic factor alone produced for the most part chromatolysis in the nerve cells only, though in the sciatic nerve of a rat which had been paralysed for 12 days there was evidence of very early degeneration. The absence of degenerative changes, recognisable by the Marchi method, in the peripheral nerve of the rat is in striking contrast to the myelin changes formed in the peripheral nerves of other mammals and of birds deprived of vitamin B₁.

(3) **In Other Mammals**—(a) **MONKEYS**.—Degeneration of the peripheral nerves has been produced in monkeys by a diet of boiled rice by Shiga and Kusama (1911), and by McCarrison (1919). More recently (1927) Kikuchi has attempted to study experimental B-avitaminosis in apes.

Among the results obtained were the following: In apes on a vitamin B-deficient diet, symptoms of both gastro-intestinal and nervous types appear. The incidence of the nervous form is delayed by administration of proteins and fats. The disturbance of digestion is manifested by loss of appetite, diarrhoea, and constipation. In the gastro-intestinal form, necrosis, and ulcers of the mucous membranes develop. In the nervous form, central nervous affections are occasionally found, leading to degeneration of the Purkinje cells. Usually the peripheral system is affected, causing paralysis. The body temperature, respiration, and pulse-rate tend to decrease slightly. The erythrocyte count and hæmoglobin content of the blood are diminished. The blood sugar and liver glycogen remain fairly normal, as does the sugar tolerance of the animal. On administering vitamin B to the apes suffering from B-avitaminosis, the central nervous symptoms, if present, are alleviated, and erythrocytes, hæmo-

globin, respiration, and pulse-rate tend to become normal. The body weight, gastro-intestinal symptoms, and peripheral paralysis remain uninfluenced by administration of vitamin B. Kikuchi concludes that the symptoms of B-avitaminosis in apes resemble, on the one hand, those of vitamin B deficiency in the lower animals and those of human beri-beri on the other, probably being more closely related to the latter.

(b) CATS AND DOGS.—Andrews (1912) produced degenerative lesions in the nervous system of puppies by feeding them on the milk of women whose children had died from beri-beri, while Voegtlin and Lake (1919) produced polyneuritis in cats and dogs in from 4 to 6 weeks by feeding them on a diet of lean beef heated for 3 hours at 120° C. in the presence of alkali. The sciatic nerves of these animals stained by the Marchi method showed all stages of myelin degeneration.

LII. FURTHER PHYSIOLOGICAL ASPECTS OF VITAMIN B₁ DEFICIENCY.

Besides the more obvious disorders contained in the beri-beri complex, lack of the anti-neuritic vitamin produces other ill effects which the modern school of vitamin research regards as of extreme importance to the health of the community. Many of these disorders, it is thought, have been long unrecognised as due directly to vitamin deficiency, because the insidious development of methods of food preparation destructive to their vitamin content has become more closely connected with vitamin B than the other vitamins. Especially important in this respect are the milling of cereals (one of the sources *par excellence* of vitamin B), and certain methods of preservation of foods at temperatures and oxidative conditions inimical to its stability. On this account the diet of the majority, until the last few years, must for some time past have been deficient in vitamin B to a degree which was sufficient to produce many of these ill-defined disorders of metabolism.

(A) ANOREXIA.—That loss of appetite does occur during vitamin B deficiency is an established fact, so well established that inanition in avitaminosis B has been attributed to lack of appetite alone. Drummond showed in 1918 that soon after the administration of a diet deficient in vitamin B the food consumption fell to a level approximately that required to supply the calorific requirements for maintenance. The stimulation of appetite by vitamin B extracts has been shown by Cowgill, Deuil, and Smith (1925) and by Sure (1928). Cowgill (1921) also found that the potency in promoting appetite ran parallel to the potency in relieving symptoms in polyneuritic animals. In human beings appetite was restored, according to the observations of Bierry and Kollmann (1928), by the administration of 100 to 150 mgrms. of vitamin B concentrate.

The increase in food intake and in weight of rats on a partially B-deficient diet when given yeast is attributed by Griffith and Graham (1929) not to the stimulation of appetite but to an increased utilisation of food. On the other hand, they consider that the further addition of liver extract results in an increased rate of growth owing to the stimulation of appetite and not to increased utilisation of food. Opinions are not unanimous as to the actual mode of action of vitamin B₁ in promoting appetite.

The following are some of the theories advanced to explain this action :

(1) **Increase of Gastric Motility.**—Experiments have been carried out by Rose and co-workers (1930) to determine whether the loss of appetite in vitamin B deficiency had its physiological basis in a disturbance of gastric motility.

By means of recording the gastric movements of dogs in which vitamin B deficiency had not been so advanced as to cause neuro-muscular symptoms, they decided that anorexia was not necessarily associated with gastric atony. The avidity for food did not seem to depend upon the vigour of hunger contractions in these experiments.

(2) **Relief of Intestinal Stasis.**—Samson Wright (1921), supporting the evidence of Lumière

(1920) and Karr (1920), suggested that vitamin B produces its effects by acting primarily, if not solely, on the alimentary canal. According to his theory, in the absence of vitamin B muscle-tone is diminished, peristaltic movements are weak, and there is a reduction in the amount of digestive juice secreted. As a result, stasis occurs, the appetite flags, and the food intake falls off considerably.

(3) **Stimulation of Endocrine Glands.**—The experiences of Bierry and Kollmann, above referred to, lead them to conclude, "mainly on theoretical grounds," that the stimulating action of vitamin B is exerted on the glands of internal secretion.

(4) **Specific Tissue Action of Vitamin B₁.**—The investigations of Kon and Drummond (1927) are in direct contradiction to the "intestinal stasis" theory. No difference in the rate of passage of a barium meal through the intestinal tract was observed in pigeons on deficient diet and normal controls. Kon and Drummond concluded that lack of appetite for the deficient food was characteristic of vitamin B deficiency in the pigeon, but could furnish no satisfactory explanation of the exact rôle played by vitamin B in this connection.

(5) **Protective Tissue Adjustment.**—The most recent theory, that loss of appetite is actually a protective reaction, is advanced by Peters (1930). He points out that there is a constant time for the induction of symptoms in rice-fed birds, and that this time is usually shortened in birds fed by stuffing. (See also Braddon and Cooper (1914), Randoïn and Lecoq (1929).) Since the occurrence of symptoms is delayed in birds feeding naturally, and since the delay is due to loss of appetite, Peters considers that the loss of appetite is a protective adjustment of the tissues against their disorganisation by vitamin B deficiency. He points out that loss of appetite is secondary to the internal starvation, and a reflection from tissue cells which have lost their rightful powers of assimilation.

(B) **LOSS OF WEIGHT.**—The loss of weight in animals fed on vitamin B deficiency is marked and progressive. In McCarrison's experiments on pigeons (1919) the loss of weight amounted roughly to about one-third of the original weight of the bird. It was due mainly to muscular wasting as well as to a thinning of the bony framework of the body. All subcutaneous fat had disappeared; only in a very small percentage of cases was slight subcutaneous œdema of the abdomen and groins observed.

The chart on opposite page shows the progressive loss of weight of pigeons fed on milled autoclaved rice as compared with normal controls fed on mixed grains.

It has been pointed out by Barlow (1926) that the descent of body-weight temperature and respiration rate in pigeons on an exclusive polished rice diet shows a curve analogous to, but more gradual than, that in fasting pigeons.

The relation between the vitamin deprivation and the loss of weight has been the source of much discussion.

It is generally agreed that the loss of weight is not in itself the cause of the illness. In forcibly-fed birds the symptoms arise more quickly with less loss of weight than in birds feeding naturally. Kon and Drummond (1927) have shown, by feeding control birds with an amount of food similar to that actually consumed by avitaminous birds, that loss of weight in birds feeding naturally is due to diminished intake of food. Peters considers that the loss of weight may be protective against a final dissolution, owing to the liberation of essential factors from tissue stores. The stemming of the loss of weight in forcibly-fed birds may therefore not be conducive to the amelioration of symptoms.

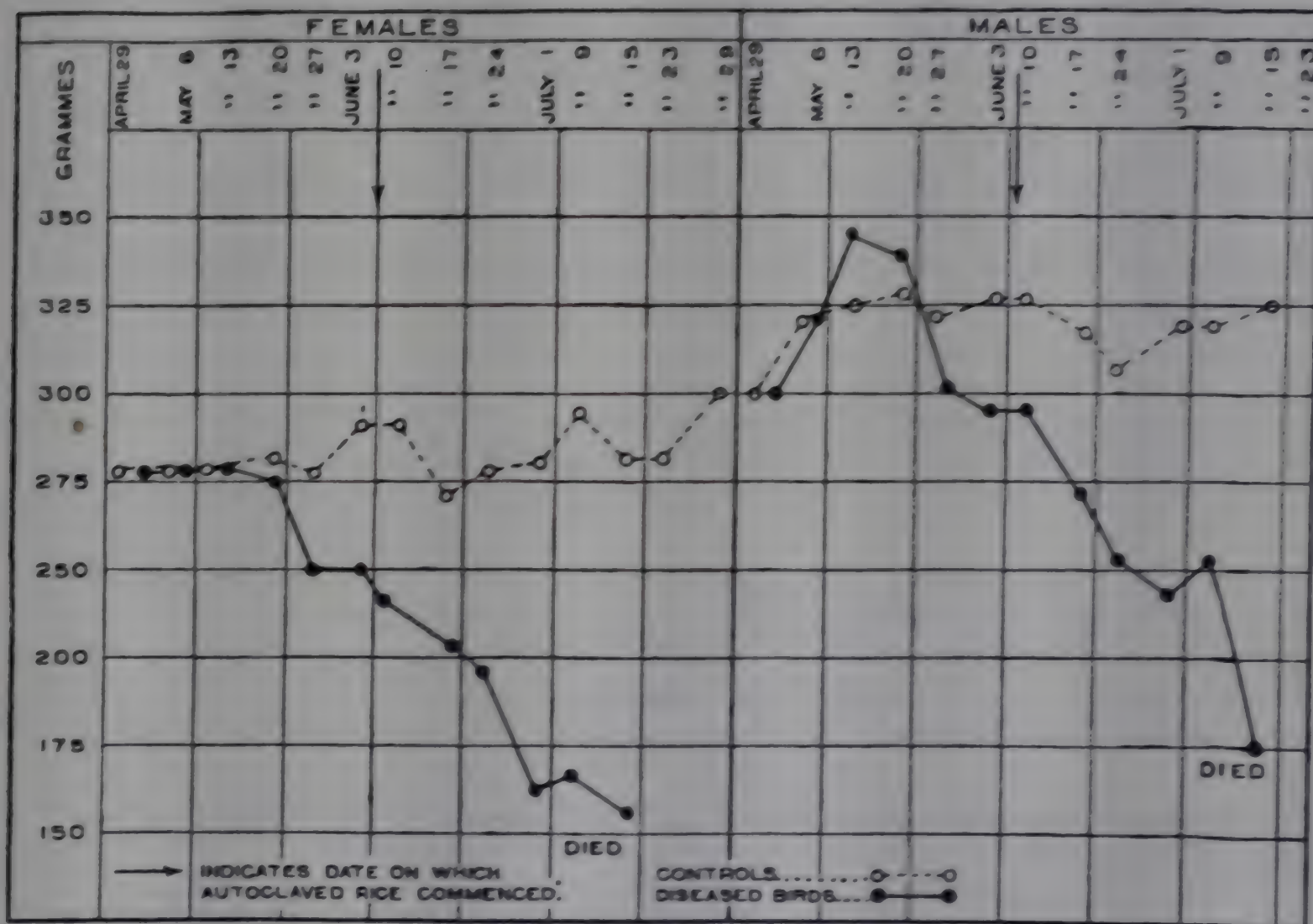
(C) **FALL OF BODY TEMPERATURE.**—A steady fall in the body temperature occurs in experimental B avitaminous animals.

McCarrison (1919) found the diurnal range of healthy pigeons from 39.8° C. to 43.2° C., the noon average of 41.6° C. In deficient animals it may be reduced to 36.6 or 37.2° C. The rectal temperatures of 10 pigeons fed on a vitamin B-deficient diet by Marshall

Findlay (1923) is shown in the chart on page 264. It will be seen that the reduction in temperature becomes more marked after the twentieth day on the diet.

Findlay attempted to correlate this fall in body temperature with the increased susceptibility to bacterial infection found in B vitamin-deficient animals. He found this reduction of natural immunity marked only when the cloacal temperature was 40° C. or below, and considered that the lowered temperature decreased the resistance by (a) facilitating the growth of the invading organisms, (b) reducing the leucocytic response to the infection, (c) reducing the bactericidal power of the leucocytic exudate.

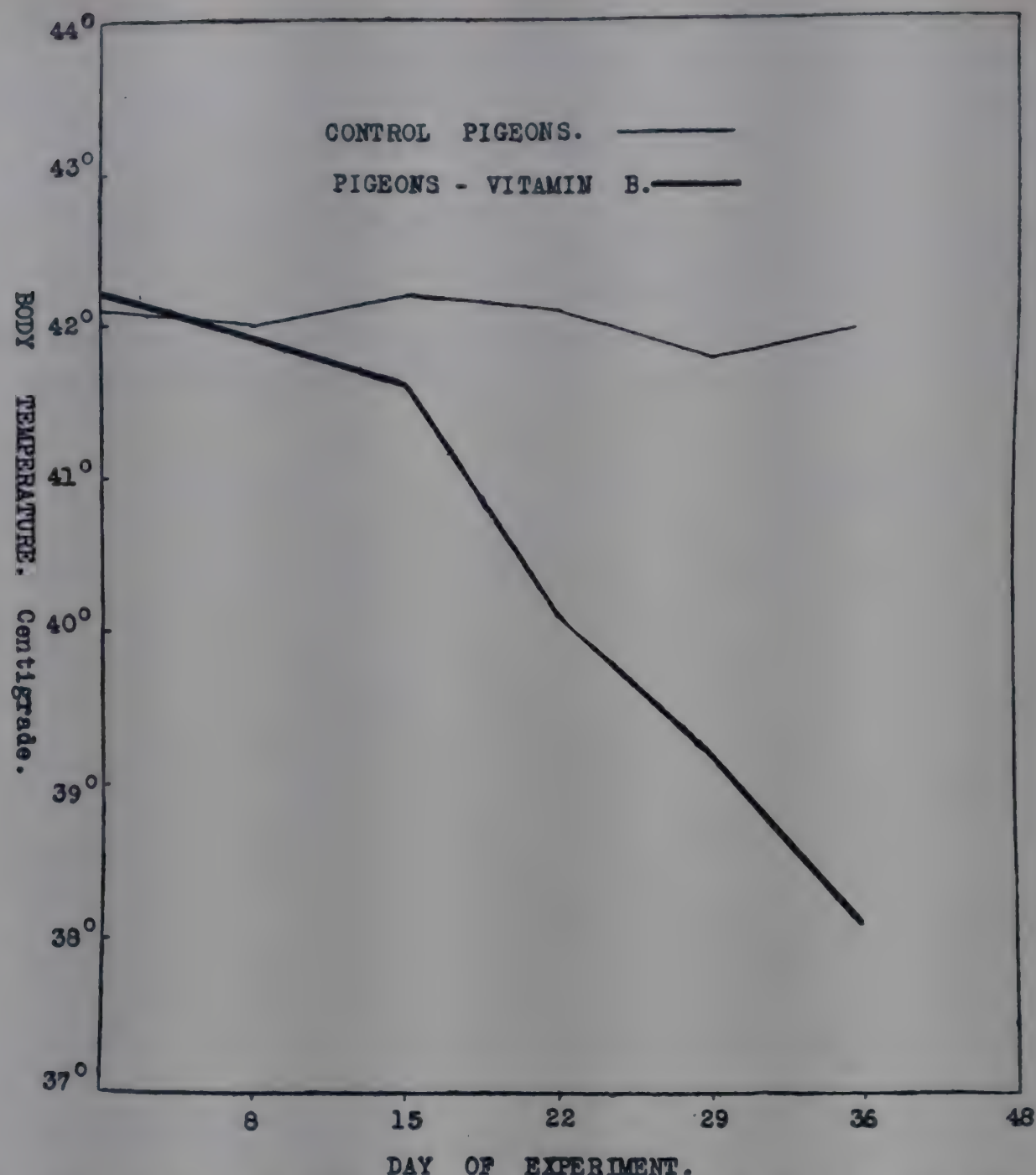
A similar fall in body temperature occurs during starvation, and a reasonable explanation seems to be that the hypothermia is a result of exhaustion of available food reserves, occurring



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either in vitamin B deficiency or inanition, and not specifically due to vitamin B deficiency.

(D) **GASTRO-INTESTINAL LESIONS.**—Many observations have been made on the changes, both anatomical and functional, said to be present as a result of vitamin B deficiency. The careful and detailed work of Gross (1924) sounds a note of warning against the too-ready acceptance of the relation between the avitaminosis and the changes observed. Gross's observations on "normal" control rats showed so many and great individual variations and such marked intercurrent intestinal lesions that it becomes increasingly difficult to assess the part played by vitamin deficiency in the occurrence of similar lesions in avitaminous animals. As an example of the varying grades of severity of inflammatory processes occurring



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in these "normal" animals (which Gross regards as representative of the experimental animals commonly used in vitamin research), the following table may be given :

PERCENTAGE INCIDENCE OF INFLAMMATORY LESIONS IN THE CÆCAL ASPECT OF THE ILEOCÆCAL REGION.

Diet.	Ulceration.	Distinct Inflammatory Infiltration.	Slight Accumulation of Inflammatory Cells.	Total.
Full	19	12	30	61
B-deficient ...	13	11	16	40

(E) **PATHOLOGICAL CHANGES IN THE ALIMENTARY CANAL.**—In Gross's cases the lesions found were slighter than those described by McCarrison (1919, 1922, 1927). Gross found :

(1) **In the duodenum** a more or less granular exudate (or transudate) containing occasional lymphocytes, and lying immediately under the epithelium of the tips of the villi (16 per cent. as opposed to 2 per cent. in controls).

(2) In the descending colon a moderate atrophy of the reticular cells of the mucosa (43 per cent. as opposed to 6 per cent. in controls).

(3) In the ileocæcal region inflammatory changes of varying grades of severity, but in the same incidence as in controls. An increased incidence of pigment-bearing cells in the sinuses of the retro-ileocæcal glands was, however, found in the vitamin B-deficient animals.

Cells somewhat similar in appearance are found in the human colon in cases of stasis.

(4) In the Peyer's patches—regressive changes—elongation and clumping of the lymphocytes, and hyaline connective-tissue stands between the clumps.

In McCarrison's animals the changes consisted chiefly of congestion of the small vessels ramifying between the muscularis mucosæ and the base of the crypts of Lieberkühn; hæmorrhages into the coats of the bowel; atrophy of the myenteron, mucous membrane, and lymphoid structures; degenerative changes in the plexus of Auerbach and bacterial invasion of the bowel walls, but to a less degree than in animals dying from complete "vitaminic" deficiency. The findings of atrophy of the bowel wall, particularly of the cæcum, and of bacterial invasion, have been confirmed by Rowlands (1927) and Browning (1927).

In the experiments of the above workers rats were kept for prolonged periods (35 to 80 weeks) on a diet only partially deficient in vitamin B (2 per cent. of marmite being given). Sections of the colon and cæcum showed marked thinning of the muscular and submucous coats.

Bacterial invasion was shown by administering a streptococcal broth emulsion (1 c.c. of a 500 million broth culture), added to milk, to rats which had been fed for 9 weeks on a diet containing 2 per cent. marmite, and for 4 days on a complete B-deficiency diet. Sections of the colon of these animals showed the presence of streptococci in the lacteals of the villi, while in the normal control animals, fed on the same streptococcal emulsion, no organisms were present.

(F) **FUNCTIONAL CHANGES IN ALIMENTARY TRACT**—(1) **Motility**.—The evidence of most workers points to the conclusion that the motor functions of the whole intestinal tract are impaired in vitamin B deficiency.

Lumière (1920) found evidence of stasis in the upper part of the digestive tract of pigeons fed on decorticated rice. Trituration in the stomach was defective, and the onward passage of the bolus through the pylorus was greatly delayed. There was deficient glandular secretion and hypotonus of the intestinal wall. A small dose of extract of rice polishings provoked abundant glandular secretion and strong intestinal movements which resulted in rapid evacuation of the masses of polished rice lying in the upper intestine.

That the impairment of motility takes place even higher in the digestive tract is shown by the observations of Inawashiro (1929) on the force of contraction of the gizzard in hens and pigeons. The force of contraction was measured by introducing a rubber ball which was filled with water and connected with a manometer. Birds fed on diets deficient in vitamin B showed at first a diminution in the contraction pressure, and, later, after neuritic symptoms had set in, a disappearance of this pressure.

Impairment of gastric motility was also found in the experiments of Cowgill and co-workers (1926). Four dogs having permanent gastric fistulæ of the "valve" type were fed artificial diets adequate except in vitamin B. At intervals the motility of the empty stomach was recorded (Carlson technic). In mild cases of vitamin B deficiency, evidenced by an anorexia, there was little if any change in the character of the gastric contractions, but in severe cases, characterised by nervous and muscular symptoms as well as by anorexia, there was gastric atony. Successful vitamin B therapy applied to such cases was associated with rapid improvement in tone of stomach musculature.

Cramer and Samson Wright (1928) have also stated that the stomach was dilated in vitamin B deficiency and that there was probably "some defect in the power of the stomach to deal with its contents and propel them farther down the intestine."

These observations were borne out by the findings of Rowlands and Browning (1928) in which X-ray photographs, $3\frac{1}{2}$ hours after a meal, of rats fed on a vitamin B-deficient diet showed marked distension and visceroptosis.

A decrease in gastric motility has been demonstrated by Stucky and co-workers (1928, 1930) by means of tracings from a gastric fistula. These workers suggest the probability that the decrease in gastric activity is not due to any specific effect of a lack of vitamin B, but a deficiency of this factor seems to accentuate the disturbance. The blood sugar was normal in all cases, so that hyperglycæmia was not responsible for the effect. The behaviour of the stomach in the control dogs suggested that the water intake may be a factor affecting its muscular contractions. Injection of insulin was followed by improvement, and this response was not due to the presence of vitamin B₁ in the insulin, since further experiments by Stucky (1928) on pigeons have shown it to be absent. From later experiments (1930), Rose and co-workers suggest that the relation of insulin to gastric motility is not a simple one. Insulin appeared to precipitate generalised neuromuscular symptoms in 2 dogs, which suggests that sensitiveness to insulin is increased in B-avitaminosis.

In the intestinal tract the impairment of motility is still more generally agreed upon.

The experiments of Gross (1924) are very conclusive. His method was to feed rats on a diet with which finely powdered animal charcoal was thoroughly mixed. The animal was then placed on a specially designed metabolism apparatus (Gross and Connell (1923)) which automatically times and separates the excreta. With this apparatus it was possible to estimate to within an hour the time taken for the first appearance and for the disappearance of the charcoal in the fæces. The average time taken for the charcoal to make its first appearance was approximately 10 hours for full as well as vitamin-deficient diets. The time taken for the charcoal to disappear completely was enormously increased in the case of vitamin B deficiency—charcoal was present in the fæces after 16 days in the B-deficient rat, whereas it had appeared in 65 hours in the control rat.

A similar delay, though not so prolonged, was shown in the experiments of Rowlands (1927) and Browning (1927) by radiographs of the intestinal canal after a barium meal. Further confirmation has been received from the experiments of Plummer (1927) on excised intestinal musculature. Excised intestinal strips from the duodenum, ileum, and colon were suspended in oxygenated Locke's solution at 38° C. Levers were arranged which recorded rate and amplitude of rhythmic contractions and tonus waves, and time during which the strips exhibited spontaneous contractions. Tracings were run from refrigerated segments at 1, 2, 3, and 4 days after death, with tracings from normal animals as controls. Absence of vitamin B from the diet of a rat diminished the time during which an excised strip exhibited spontaneous contractions. Amplitude and rate of rhythmic contraction were markedly decreased in the spastic stage of beri-beri rats. The tonus of the intestinal musculature was also lessened. Segments from the beri-beri intestine placed on ice for 24 hours showed no spontaneous contraction or tone, whereas normal strips were active on the second day. Plummer concludes that constipation and decreased motility of the intestinal tract are characteristic of rats suffering from beri-beri.

(2) **Secretory Function.**—The pronounced necrotic changes in the secretory elements of the entire gastro-intestinal tract (gastric and pyloric glands, glands of Lieberkuhn, etc.) observed by McCarrison (1919) are evidently such as to cause grave disturbance of the digestive and assimilative functions. The fact that these lesions were noted in extreme cases of vitamin deficiency, of which vitamin B was not the sole factor, does not detract from their importance as evidence of the type of change brought about by lesser degrees of vitamin B deficiency. Diminution of the total volume and of the free and total acidity of the gastric secretion has been shown by Farnum (1926) to occur in experimental beri-beri. Skarzynska-Gutowska

(1928) has stated that intravenous injections of vitamin B extracts induce a flow of gastric juice about one-sixth of that produced by injection of 0.5 mgrm. of histamine. That food rich in vitamin B contains substances which stimulate the flow of bile and pancreatic juice was shown by Voegtlin and Myers (1919). In Gross's experiments also the fæces in vitamin B deficiency were grey and somewhat lacking in bile pigments, and there was a diminution in the number of scybala passed, which returned to normal when "marmite" was administered. Cramer and Mottram (1926) have shown that the vitamin B content of the food greatly modifies the food absorption from the intestinal canal, and that substances rich in vitamin B have an intensely stimulating effect on the functional activity of the whole digestive tract.

Bergeim (1928) found a reduction in protein digestion on a B-deficient diet, confirming the earlier findings of Farmer and Redenbaugh (1925–1926) of a decrease in the enzyme content of the pancreas and upper intestine in polyneuritic pigeons. An increase in the amount of intestinal secretion has been stated by Kawamura (1929) to occur when vitamin B in the form of rice bran extract is administered to animals suffering from B vitamin deficiency.

(3) **pH Concentration of the Intestinal Canal.**—A determination of the pH concentration in different parts of the intestinal canal in rats on normal and B-deficient diets was made by Schoubye (1928). The animals were anaesthetised and the secretion from an isolated portion of the intestine was collected into a petri capsule, the pH concentration being determined by electrometric and by colorimetric methods. In all the animals throughout the greater part of the intestinal canal the contents were acid, but there was a rise in the pH towards the last part of the ileum, the values varying from pH 7 to pH 8. The animals showing signs of vitamin B deficiency, however, showed the highest alkalinity in the cæcum, after which the pH value fell in the upper part of the large intestine to that found in the other groups, namely, pH about 6 to 7.

(G) **CHANGES IN THE ENDOCRINE AND OTHER ORGANS.**—That the endocrine organs show very definite alterations in vitamin B deficiency is the general opinion of practically all workers, beginning with the extensive investigations of McCarrison in 1919.

McCarrison showed that certain organs undergo hypertrophy, others atrophy. Those which hypertrophy are the adrenals. Those which atrophy, in the order of severity named, are the thymus, testicles, spleen, ovary, pancreas, heart, liver, kidneys, stomach, thyroid, and brain. The pituitary gland showed in adult birds a slight tendency to enlargement in males only.

McCarrison regarded the disturbance of function as due in part to the nuclear starvation of the organs composing the endocrine system, in part to the failure of efficient sympathetic control, and in part to disturbance of their correlation.

That the symptoms of avitaminosis are not due to a direct lack of secretion caused by the B vitamin deficiency appears to be proved by the experiments of Borchardt (1927). He obtained negative results when he attempted to influence the progress of symptoms of polyneuritis in pigeons by giving specific hormones. Neither dried internal secretory gland substances nor extracts prepared from the glands were preventive or curative. The symptoms of beri-beri could not be reproduced in other dogs, receiving normal diets, by removal of the thyroid or adrenals or pancreas or other glands of internal secretion.

(1) **The Adrenals.**—Hypertrophy of the adrenals in vitamin B deficiency is so marked and incontrovertible that some workers, especially Bierry, Portier, and Randoin-Fandard (1920) have regarded it as the key to the physio-pathology of polyneuritis. Funk (1919) regarded it as the cause of the hyperglycæmia which he and Schonborn first noticed, while Cramer (1920) thought the lowered temperature in vitamin B deficiency might be due to interference with the heat-regulating function of the adrenal. McCarrison has also laid great emphasis on the relation between adrenal hypertrophy and the genesis of oedema. The fact that a similar

hypertrophy occurs in inanition has tended to throw doubt upon this hypothesis, while Peters' observations on the "anti-œdema" factor of torulin have reopened the question of the production of œdema.

In McCarrison's (1919) experiments the enlargement of the adrenals in pigeons was remarkable. They were swollen, prominent, and of a yellow-red or reddish-brown colour, often hyperæmic, diffuent, and easily torn, frequently weighing less than their size would suggest. Much of the yellow ochre colour of health had disappeared or could only be found in patches.

The actual increase in weight is shown by the following figures (from McCarrison) of the average weights of adrenals in normal and diseased pigeons:

	CONTROLS.		B VITAMIN DEFICIENCY BIRDS.	
	Male.	Female.	Male.	Female.
Average weight in mgrms. ...	29.2	24	42	41.9
Average weight per kilo of body-weight in mgrms. ...	100.3	90	139.5	149

Other workers, however, including Vincent and Hollenberg (1920), Findlay (1921), Marrian, Drummond, and co-workers (1927), and others have not found this hypertrophy so constantly present in B vitamin deficiency as in inanition. Gross (1923), for instance, found that pure vitamin B deficiency in the rat causes very little alteration in the relationship between body weight and adrenal weight unless the animal was brought to the point of death on the diet. He considered that the striking changes recorded by other observers were probably produced by the accompanying general starvation and unbalanced dietary, and were not due to vitamin deficiency as such.

Later work by Marrian and Parkes (1928), however, confirms previous results, showing that inanition and vitamin B deficiency each and separately result in adrenal hypertrophy. The forcibly-fed vitamin B-deficient pigeons showed little or no loss of weight when the typical symptoms occurred. Adrenal hypertrophy was shown by each of 2 groups of birds fed in a similar manner but which were respectively receiving extracts containing the thermostable vitamin B₂ and the antineuritic vitamin B₁. The adrenal hypertrophy was considerably greater in the case of the group deficient in vitamin B₁. Of the total hypertrophy, 19 per cent. is accounted for by œdema in vitamin B-deficient pigeons, and 44 per cent. in the case of the starving birds.

(a) HISTOLOGICAL APPEARANCES.—Most workers have found the enlargement chiefly in the cortex, though Houlbert (1919) localised the lesions chiefly in the medulla. McCarrison found the cortical columns in greater proportion in the section in diseased than in healthy birds. This observation was confirmed by Verzár and Peter (1924), who found the proportion of medulla to cortex 1/9 in normal, 1/14 in B-deficiency rats.

McCarrison and Parkes (1928) stated that the enlargement was mainly in the cortex in the B-deficient birds and in the medulla in starvation.

The chief cellular changes found by McCarrison were: (i) The nuclei of both medulla and cortex were fewer in number, area for area, in the diseased cases. (ii) The nuclei often appeared more vesicular, larger, and frequently more shadowy in form, while cells without nuclei, or containing only shadow nuclei, were commonly met with. (iii) Some cells of the cortex showed small uniformly dark-staining nuclei which were in marked contrast to the more vesicular nuclei of the normal cortex. (iv) Some diseased glands showed many cells with brightly staining eosinophile granules when stained by Mann's stain. (v) In the sympathetic nervous tissue, sometimes found adherent to the periphery of the gland, some of the large cells of the

ganglia presented appearances (changes in the nuclei and diffuse starving of the cell body) indicative of degeneration.

Kellaway (1921) described an increase of lipoid content, less definitely granular than normal and a nuclear degeneration. It is stated also by Lasowsky and Simnitsky (1926) that the cortical hypertrophy depends on increase of fats (among which the mixture of cholesterin esters with neutral fat plays a prominent part) infiltrated from the blood stream.

According to these workers, alterations of cortical cells represent a secondary reaction to fat saturation. Part of the cells are destroyed; and later, proliferation is observed, karyokinetic figures appearing in almost every field. Hypertrophy and hyperplasia thus coexist. They believe that hypertrophy of the adrenal cortex is not specific for vitamin B deficiency, but has been found in other conditions (pregnancy, castration, hunger) wherever a rise in fat content of blood is observed.

In man, Bernard and Bablet (1925) have shown the chief lesions to be those of hæmorrhages in both cortex and medulla, causing disorganisation of the cellular columns. The cells themselves showed no signs of degeneration.

(b) CHANGES IN ADRENALIN CONTENT.—In starvation the adrenalin content of the adrenals is increased proportionately to the hypertrophy. Whether this is the case in B-vitamin deficiency is apparently a matter of dispute. McCarrison has stated that the enlargement of the adrenals is a true hypertrophy accompanied by a great increase of the epinephrine content of the organs. He based his statement on the evidence obtained from kymographic observations of the arterial blood pressure in sheep. The adrenals, from healthy and diseased birds respectively, were weighed, and emulsified in a sterile mortar with saline solution. The emulsions were injected into the left jugular veins of sheep under ether anaesthesia, the kymographic tracing being taken from the right carotid. As a control, $\frac{1}{16}$ mgrm. of adrenalin was injected in the same way.

The results of these observations were as follows :

- (a) 20 mgrms. of healthy adrenals caused a rise of blood pressure of 21 mm. of Hg in 38 lb. sheep.
- (b) 50 mgrms. of beri-beric birds' adrenals caused a rise of blood pressure of 58 mm. of Hg in 40 lb. sheep.

McCarrison also stated that cases of beri-beri with œdema had larger adrenals, containing more adrenalin, than cases without œdema. He recognised the fact that absolute proof of hyperadrenalinæmia can only be afforded by demonstrating an excess of adrenalin in the venous blood from the adrenals, yet he believed the above evidence justified the presumption that adrenalin entered the blood stream in excess. Other workers, however, including Gross (1923), Marrian, Drummond, and co-workers (1927), have found neither an increase in adrenalin content nor a hyperadrenalinæmia.

Kaufman (1923) and Beznak (1923) even state that there is a diminution in the content of adrenalin in pigeons.

It is as yet uncertain what is the exact significance of the adrenal hypertrophy, but as Drummond (1926) suggests, the solution may be along the lines indicated by the researches of Cannon, McIver, and Bliss (1923), who demonstrated that any condition tending to cause a reduction of the blood sugar level will lead to increased output of adrenalin.

(2) **The Thyroid and Parathyroids.**—The thyroid decreases in weight in vitamin B deficiency, but its histological appearances differ very slightly from the normal gland. McCarrison found the thyroid amongst the least affected of all organs by the deficient dietary. The changes attributable to this cause consisted in mild or moderate degrees of congestion and in necrobiosis of a relatively small proportion of the secreting cells. Most workers, including

Drummond (1918), Findlay (1921), Simonnet (1921), and Souba (1923), are in agreement with these observations, but in the rat Gross has observed a greater frequency of the interalveolar epithelial cells, and a more marked secretory activity of the vesicles. An increase of secretory activity had also been stated to occur by Satwornitskaya and Simnitsky (1927) in the earlier stages of deficiency. In their experiments on rats the follicles were enlarged, lined with columnar cells, and contained much colloid. In the later stages there appeared a bursting of follicles which allowed the colloid to flow out into lymph and blood vessels. In the last stages there were some evidences of decreased activity of the thyroid in rats, but these were slight, and were not observed in pigeons.

The Parathyroids.—According to Findlay (1921), Korenchevsky (1923), and McCarrison, there is no macroscopic abnormality in the parathyroids.

(3) *The Thymus.*—A marked diminution in the size of the thymus in vitamin B deficiency has been constantly observed since Funk first drew attention to it in 1914, but its specificity with regard to avitaminosis is not undisputed.

McCarrison stated (1919), as a result of his observations on pigeons :

“In diseased birds the thymus atrophies out of all proportion to the body weight. In males its average weight is reduced to 14 mgrms. The merest traces only (and these unweighable) could be found in 13 of the diseased birds ; in the remaining 7 birds, in which a weighable thymus was dissected out, the weight of the organ ranged from 15 to 85 mgrms. In diseased females, on the other hand, the gland had completely disappeared in the present series, or existed only as a thin thread of tissue closely applied to the great vessels of the neck.”

He confirmed these observations in experiments on monkeys, and further confirmation was received from Findlay (1921), Gross (1924), Lomba (1923), Korenchevski (1923), and others. The involution which the thymus undergoes during development, and the lack of its vitamin content, as pointed out by Williams and Crowell in 1915 ; the variability of thymic atrophy in infantile beri-beri, as shown by Andrews (1912) and Miura (1899) ; and its atrophy on a diet poor in proteins observed by Findlay (1921), throw doubt upon its specificity.

(4) *The Sex Glands*—(a) *THE TESTES.*—There has been the same question of relationship between starvation and atrophy of the testis as in the effects of B vitamin deficiency on the other endocrine organs. Findlay, for instance, has pointed out that atrophy of the genital organs occurs both in animals fed on polished rice and in starving animals, whether vitamin B is present or not, and Bierry and Kollmann (1928) consider that the severe lesions reported by other observers, especially Portier (1920), have been due to prolonged inanition on a diet which was not well equilibrated, and not primarily to a vitamin B deficiency. The experiments of Marrian and Parkes (1928), however, show that the testicular degeneration observed in pigeons fed a vitamin B-deficient diet is not purely a starvation effect, since birds forcibly fed this diet may show severe degenerative changes at a time when the loss of body weight is negligible. Also pigeons dying from inanition but receiving vitamin B failed to show such severe degeneration. A control group of birds forcibly fed the deficient diet, but which were also receiving vitamin B, showed histologically normal testes.

There is a constant and pronounced diminution in size and weight of the testes in B vitamin deficiency, and the observations of Marrian and Parkes (1928) have led them to conclude that it is vitamin B₁ which is the factor concerned, vitamin B₂ being comparatively unnecessary for testis nutrition.

McCarrison (1919) found the average reduction in size about one-eleventh of their original volume, this proportion being maintained when the weight of the organs is calculated per kilo of body weight. Other observers, including Findlay (1921), Souba (1923), Gotta (1923), etc., have found a reduction varying from one-fifth to one-tenth of the original volume.

Histological Changes.—The original description of McCarrison (1919) has been little altered

by the observations of later workers, though Korenchevsky (1923) stated that he found a tendency to hypertrophy and hyperplasia of the inner secretory interstitial cells ; while Bierry and Kollmann (1928) found the connective and interstitial tissue unaffected.

The most important change is the suppression of the function of spermatogenesis. The capsule of the gland and the intertubular trabeculæ are greatly thickened, the number and diameter of the tubules are much lessened, very often the spermatids are degenerated, and the spermatocytes may be entirely absent. The tubules are lined by a single incomplete layer of cells, many of which still preserve nuclei apparently capable of regeneration.

Bierry and Kollmann have indeed stated that the lesions are not permanent, since pigeons, cured of polyneuritis, showed perfectly normal testicles when killed twenty days after cure.

Hypertrophy of the tunica media of the artery to the testis is described by Vanni (1927).

It should be mentioned that Mattill (1927) attributes the degeneration of germinal epithelium in the rat testis, as described above, not to a specific vitamin B deficiency but to simultaneous or previous lack of vitamin E. In his experiments, 13 mature normal rats showed sterility within 3 weeks after complete withdrawal of vitamin B ; at death, 4 to 8 weeks later, only 1 showed testicular degeneration. Of 14, dying in 12 to 22 weeks under gradual vitamin B starvation, only 1 had degenerated testes. Many of these, in addition to weight loss (up to 50 per cent.), showed typical paralysis ; but the epididymis still contained sperm as motile as those of normal animals.

Mattill believes that the sterility following soon after vitamin B withdrawal is due to loss of vigour and to lowered sex expression consequent upon a lowered metabolic level.

Evans (1928) also considers that acute or chronic deficiency of the antineuritic vitamin does not affect the anatomical and functional integrity of the male germ cell of the rat. It has a definite effect on sex interest, decreasing this, but an effect which may not be shown until a few days preceding death. Provided vitamin E is adequately high, injury to the reproductive function in the male caused by inadequate vitamin B would appear to be due solely to this interference with behaviour, and not to actual injury of the germinal elements.

(b) **THE OVARIES.**—The ovaries undergo a marked atrophy, to one-third of their volume, according to McCarrison's figures. Gross (1924) found many follicles degenerated, and Korenchevsky (1924) observed a hyperplasia of connective tissue. Findlay (1921) found that the membrana granulosa was composed of a single layer of epithelial cells, and that some of the interstitial cells showed degenerative changes. A marked reduction in the lipoid content of the ovary is stated by Dulzetto (1927) to occur during polyneuritis. According to his observations the granular lipoids in the interstitial cells of the ovary appear to collect into larger droplets which lose their affinity for Sudan III, being transformed into a substance staining with hæmatoxylin. The lipoid content is thus greatly reduced. Nuclei of the interstitial cells progressively lose their staining capacity and finally disappear. During inanition (on water alone) the changes are quite different. The lipoid granules of the interstitial cells persist in abundance even above the normal, and stain distinctly with Sudan III. The nuclei also persist, though reduced in size and in chromatin content.

Œstrous Cycle.—In 1913 Vedder pointed out that women suffering from beri-beri cease to menstruate, the function only returning during convalescence.

Although it is generally agreed that deficiency of vitamin B affects the œstrous function there appears to be some difference of opinion as to the exact change produced.

Reiss and Perény (1928) state that "lasting heat" is produced, which is apparently independent of the ovary, since it takes place also in ovariectomised animals.

According to Parkes (1928), on the other hand, a deficiency of vitamin B, represented by 1 per cent. or less of yeast extract added to a basal diet, brings about an abrupt cessation of the œstrous cycle in rats in about 4 weeks. The anœstrus so produced, which begins just

before the characteristic decline in weight appears, applies both to ovarian and extra-ovarian phenomena of œstrus, and is terminated at any time up to 2 months later by death. He states that injection of œstrin (10 m.u.) during the anœstrous state results in induction of œstrous symptoms in the accessory organs, without, however, causing the ovary, now much atrophied, to ovulate. It is concluded that anœstrus due to this deficiency results not from inability of the animal to respond to the œstrous-producing stimulus, but from failure to produce œstrin.

In connection with the theory supported by Drummond that many of the effects attributed to vitamin B deficiency are common to inanition and not specific to avitaminosis, it is interesting to note that Martino (1927) describes an injury of germinal and endocrine function in hens suffering from acute and chronic starvation which could be brought back to normal by increasing the food supply. A lengthening of the œstrous cycle in rats is also reported by Lee (1926) as occurring in low environmental temperatures. The results are interpreted as due to the direct effect of temperature on ovarian activity and to the lowered general metabolism of these rats, shown by the lowered body temperature and lessened activity.

(5) **The Pituitary.**—The changes in the pituitary are comparable to those found in the thyroid, showing little variation from the normal. McCarrison observed a slight increase in weight, due, he considered, to moderate congestion, while Korenchevsky found an increased number and hypertrophy of the "large light cells," which, however, he would not consider characteristic of the deficiency without further confirmation.

(6) **The Spleen.**—The spleen is, according to McCarrison, always atrophied to a degree scarcely observed in any other morbid process. According to Findlay, however, the condition of the spleen would seem to depend on the chronicity of the condition. Scheube (1884) and Yamagiwa (1899) both refer to the spleen as either normal or increased in size, while Musgrave and Crowell (1922) could find no characteristic change in the spleen other than that due to passive congestion.

Histological Changes.—According to McCarrison the chief changes observed were: (1) thickening of the capsule and contraction of the organ. (2) Atrophy of splenic pulp and partial disappearance of lymphoid cells. McCarrison states that the lymphoid cells and the cells of the pulp are involved in a process of necrobiosis and absorption; they are greatly reduced in numbers. The nuclei lose their staining characters, a gradual karyolysis occurs, and the cytoplasm ultimately disintegrates. In his cases the more atrophic spleens showed an almost complete disappearance of the Malpighian corpuscles. In Korenchevsky's experience, however, there was often an increase in size and frequent hypertrophy and hyperplasia of cell tissue. (3) Increased deposit of pigment, possibly only relative, the same quantity being collected into the smaller compass of the contracted organ. (4) Thickening of the walls of the arteries. The vessels are involved in a process of proliferative arteritis often leading to great thickening of their walls and to constriction of their lumen.

In man, according to Bernard and Bablet (1925), the picture is very different. The spleen is hypertrophied, the capsule sclerosed, and the fibrous trabeculæ thickened. The Malpighian corpuscles are prominent and constitute the greater part of the organ; the pulp is rich in blood-forming cells and macrophages, and there is no reduction in the lymphoid elements.

(7) **The Pancreas.**—The changes in the pancreas are less marked than would be expected from the state of chronic inanition found in beri-beri. Some workers, including Funk and Douglas (1914), Gross (1914), and Simonnet (1921) have found it normal; McCarrison found a fairly constant and definite loss of weight.

The histological changes are both inconstant and indefinite. According to McCarrison the islets of Langerhans are normal in size and structure, but Bierry and Kollmann found them enlarged and increased in number as compared with the normal. With the exception of a

few cases in which intense hæmorrhagic infiltration of the organ had occurred, or in which necrosis of the peripheral alveolar cells was marked, the pathological changes in the pancreas, as reported by McCarrison, were not such as to imply great derangement of its function.

(8) **The Liver.**—The liver undergoes atrophy to some extent masked by the passive congestion, which is a marked feature.

(a) **HISTOLOGICAL CHANGES.**—The most marked change found is passive congestion, with which are associated necrobiosis of the liver cells and karyolysis in the moderate degrees of severity, extensive necrosis in cases complicated by infection.

In Findlay's cases (1921) cloudy swelling alone was noted in a few instances, but in the majority there was extensive fatty degeneration, also observed by Emmett and Allen (1921) and Voegtlin and Lake (1919).

In man, according to Bernard and Bablet (1925), there occurs a leucocytic infiltration and extravasation of blood cells. Areas of fatty degeneration are found round the intra-lobular veins.

(b) **GLYCOGEN FUNCTION.**—The conflicting evidence on the diminution of glycogen in the liver has arisen from the simultaneous complication of starvation. In the findings of Eggleton and Gross (1925), Collazo (1923), and Handel (1924), for example, the glycogen content showed a marked fall, but they were using animals feeding naturally. In cases of avitaminosis uncomplicated by starvation, according to Randoin and Simonnet (1927), the glycogen content is not reduced.

(c) **EXCRETORY FUNCTION.**—According to Saiti (1929) there is less disturbance of the excretory function of the liver in vitamin B than in vitamin A deficiency. It was examined by injecting organic dyes (azorubin and erythrosine B) into the blood stream and determining the amount of the dye in the bile after certain time periods. The amounts excreted in the first 2 hours were less than in the control birds.

(d) **OXYGEN CONSUMPTION.**—The oxygen consumption of the liver is considerably reduced according to Tsukamoto (1928). In his experiments, pigeons were fed on polished rice, and after about 2 weeks, when symptoms of avitaminosis appeared, the birds were bled to death and the liver removed aseptically. The liver was then cut into thin sections, placed in respiration flasks in the incubator, and the O₂ consumption measured by Warburg's method.

(e) **LECITHIN FUNCTION.**—According to Naito (1923) the liver of the avitaminotic bird fixes lecithin less easily than the normal.

(f) **CHOLESTEROL FUNCTION.**—According to Asada (1925) the liver of the avitaminotic bird fixes a definite quantity of cholesterol, while the original amount remains unchanged in the normal bird.

(9) **The Kidneys.**—In animals the changes in the kidney are not so marked as to impair its functional capacity.

Atrophy is very slight, according to McCarrison and Findlay, but more marked according to Souba (1923), while Wierzchowski (1924) reports a final increase in weight following an initial diminution. Vanni (1927) has observed a degeneration which ends in necrosis of the canalicular epithelium, while the glomeruli remain uninjured. He ascribes this lesion to the elimination of toxic substances. Some degree of congestion is an almost constant feature, sometimes slight and confined to engorgement of the vessels, but more often involving the glomerular tufts and giving rise to intertubular extravasations.

The cellular changes are slight, some cloudy swelling being the principal lesion.

In man, according to Bernard and Bablet (1925), the renal changes are more marked, consisting of vascular dilatation, hæmorrhages, and sclerosis, sometimes, but rarely, extending to the glomeruli.

(H) **CHANGES IN THE BLOOD**—(1) **The Blood Vessels.**—In the arteries a hypertrophy of

the middle and adventitious coats has been described by Yamagiva (1889) and Findlay (1921). In the veins, McCarrison (1919) has found congestion and thrombosis.

(2) **The Blood Cells.**—(a) **THE RED CORPUSCLES** apparently undergo little change. McCarrison (1919) stated that they were diminished by about 20 per cent., but other workers, including Gasperi (1926) and Soldani (1929) have found no significant change in number. Fragility of the red cells in the early stages of avitaminosis, followed by an increased resistance in the fully developed disease has been observed by de Mare and Brancato (1929), slight polychromatophilia by Gasperi, and the appearance of young red cells by Biondo (1922).

(b) **THE LEUCOCYTES** are reduced in total number, according to some workers, though this reduction, as pointed out by Cramer, Drew, and Mottram (1921) only affects the lymphocytes. The differential count is stated by the majority of observers to show a definite increase in the polymorphonuclear and basophil cells. Findlay (1920) and Happ (1922), however, have failed to find any change in the differential leucocyte count.

(3) **Hæmoglobin.**—An increase in the hæmoglobin in the later stages of B vitamin deficiency has been stated to occur by Stucky and Rose (1929). The anhydræmia which this increased hæmoglobin denotes is regarded by these workers as largely attributable to the incidental starvation. A reduction in the water intake to the extent of 13 to 18 per cent. per 100 grms. of body weight has been observed by Sure, Kik, and Walker (1929).

(4) **Blood Urea.**—An increase in the urea content in beri-beri was shown by Yoshikawa and Nemoto (1917), the increase being proportional to the severity of the case.

(5) **Opsonic Index.**—A considerable and progressive diminution of the opsonic index for anthrax bacillus has been observed by Biondo (1922).

(6) **Changes in Blood Chlorides.**—Stucky and co-workers (1930) report a definite hypochloræmia during the greater part of the deficient period followed by a marked increase in the chlorides during the first week of the recovery period. Starvation and reduced plasma volume can well account for this result.

(I) **CHANGES IN THE BONE MARROW.**—The chief lesion observed is that of congestion and subsequent hæmorrhage from the capillaries. This lesion, first noticed by McCollum and co-workers (1922) in rats, is found also in guinea-pigs and pigeons, and is similar to that occurring in guinea-pigs with acute scurvy. The addition of vitamin C in the form of orange juice, however, does not prevent it.

According to Findlay (1923) there is a little reduction in the leucoblastic elements except when the birds have survived for a long period on a diet not wholly lacking in vitamin B, when a condition of gelatinous degeneration occurs, the leucoblastic cells then undergoing reduction. McCarrison (1919), however, states that there is loss of bone marrow, while Randoin and Simonnet (1927) consider that there is definite diminution of the cellular elements, not the result of the hæmorrhage, but preceding it.

(J) **CHANGES IN THE LYMPHOID TISSUES.**—The experiments of Cramer, Drew, and Mottram (1921) suggested that atrophy of the lymphoid tissue was a constant and essential result of vitamin B deficiency, but Findlay (1923) points out that it is not seen in every rat fed on a diet complete except for vitamin B nor in all cases of human beri-beri.

(K) **CHANGES IN THE BONY AND MUSCULAR TISSUES.**—(1) The *bones* of birds fed on polished rice, examined by Findlay (1921), had apparently taken part in the general atrophy and were lighter than normal.

Parino (1926) states that a diet consisting exclusively of polished rice, deficient in vitamin B, greatly reduces and retards regeneration of bone in pigeons, though not sensibly diminishing nor delaying the production of new connective tissue.

(2) The *muscles* in Findlay's observations were soft and rather dark in colour, showing some atrophy and loss of cross striation. The later finding was confirmed by Kaufman

(1923), who observed also a tendency for the fibres to appear as isolated longitudinal elements.

A study of the pectoral muscles of the vitamin B-deficient pigeons by Larcher (1927), however, showed no retardation of new-formed muscle fibre development, the degenerative process going on with almost the same intensity in the avitaminotic as the normal birds.

(L) **RESISTANCE TO BACTERIAL INFECTION.**—It is fairly generally agreed that vitamin B deprivation is associated with an increased susceptibility to bacterial infection, though recent experiments by Lassen (1929) would seem to contradict this belief. Thus, McCarrison (1919) found that hens fed on polished rice were more readily infected with *B. ærtrycke* than control birds (Lassen (1929) has failed to confirm this observation in mice), and he has also stated in connection with beri-beri that the vitamin deficiency renders the body very liable to be overrun by a rank growth of bacteria.

Biondo (1922) and Werkman (1922) have shown that pigeons on a vitamin B-deficient diet are no longer immune to infection with anthrax bacillus.

Findlay (1923) has shown a diminution of resistance to pneumococcus, meningococcus, *B. coli*, and *B. enteritidis* (Gaertner).

Special reference to the connection between vitamin B and immunity to *B. Welchii* was made by Rose (1926) during the course of an investigation in which severe symptoms of vitamin B deficiency in a dog were cured by immediate parenteral injection of a vitamin B concentrate. An abscess at the site of injection, with *B. Welchii* as its causal organism, recovered rapidly under doses of material rich in vitamin B, and repeated cultures from the site of the wound proved to be negative for *B. Welchii*. Normal animals, inoculated with cultures of this organism, showed no untoward symptoms, while those fed on a diet deficient in B vitamin gave positive blood cultures, which subsequently became negative under vitamin B therapy.

According to Findlay, while the opsonic power of the blood and the capacity to form agglutinins, hæmolysins, bacteriolysins, immune body, and antitoxin remain unimpaired, it is usual to find a fall in the bactericidal power of the blood in animals fed on diets deficient in vitamin B.

This fall in bactericidal power has also been observed by Smith and Wason (1923) with regard to *B. typhosus*, and a diminution of phagocytic power for staphylococcus by Werkman (1923) and Findlay and MacKenzie (1922).

Werkman also found that the absence of vitamin B from the diet of rats did not influence the production of agglutinins, precipitins, hæmolysins, or bacteriolysins. Similarly pigeons lacking vitamin B, or pigeons on a diet of polished rice, elaborated agglutinins for the typhoid bacillus. Experiments by Marinelli (1927), however, indicate that pigeons on a diet of polished rice injected with typhoid antigen yield much less agglutinin than pigeons on normal diet. According to his observations, in the serum of the experimental pigeons, no agglutination is apparent after 2 hours at 37° except in the higher concentrations of serum. In greater dilutions, agglutination is apparent only after 24 hours at room temperature. In pigeons on normal diet and immunised, agglutination occurs in the first 2 hours at 37° at almost any dilution. The agglutinating power of the serum of immunised pigeons diminishes rapidly in those on vitamin-free diet, while it is maintained for a long time in those on normal diet.

Attempting to explain the mechanism of the lowered resistance to infection, Findlay (1923) suggested a correlation between the fall in bactericidal power of the blood and the fall in body temperature which occurs as the result of vitamin B-deficient diets. While the temperature of the control birds varied between 41·6° and 42·4° C., that of the pigeons fed on the vitamin-deficient diet was only 38·4° to 39·8° C.

Animals with reduced body temperature lose their resisting power to certain bacterial infections—a fact first demonstrated by Pasteur and Joubert (1878), who found that if chickens

are kept on ice for some hours they become susceptible to infection with anthrax. Under normal conditions pigeons are totally immune to infection with the pneumococcus and meningococcus, but if their body temperatures are reduced to below 40° C., either by the action of some antipyretic drug or by feeding on a diet deficient in vitamin B, then the intraperitoneal injection of pneumococcus or meningococcus is followed by a fatal septicæmia.

Werkman and co-workers (1923, 1924) also considered that any differences in favour of the control animals in opsonic index were due to differences in temperatures, since in their experience vitamin-starved animals sometimes showed as much as 10° F. lowering of temperature.

The mechanism of the relation between temperature and resistance is not quite clearly determined.

The normal body temperature of the pigeon would seem to be the main factor in maintaining the natural immunity to the pneumococcus and meningococcus because of its inhibitory action on their growth; the reduction in the amount of the leucocytic exudate, and possibly also the decreased bactericidal action of the peritoneal exudate, caused by the reduction in temperature, may also be important factors.

Cramer (1922) points out the distinction between the resistance to spontaneous infection and the resistance to experimental infection. Animals that have been reduced to a state of severe malnutrition by lack of vitamin B practically never develop infections spontaneously. They are, however, more susceptible to infections produced by the injection of bacteria subcutaneously, owing to a weakening of their humoral defences against infection, and possibly also to the fall of temperature.

(M) RESISTANCE TO TOXIC SUBSTANCES.—According to Vercellana (1928), guinea-pigs fed on polished rice and showing signs of beri-beri were found to be much more sensitive to subcutaneous injections of strychnine nitrate and of aqueous extracts of poisonous fungi than healthy controls kept on a normal diet.

(N) PATHOLOGICAL LESIONS IN THE NURSING YOUNG.—A tendency to hæmorrhage in the young of animals fed on a vitamin B-deficient diet has been observed by Moore, Brodie, and Hope (1927), and by Sure and Schilling (1928). In the experiments of Moore and Brodie, albino rats raised on a synthetic basal diet containing 2 per cent. desiccated yeast as the sole source of vitamin B showed normal growth and reproduction, but lost 72·9 per cent. of their 233 young before the end of the nursing period. At a subsequent delivery these rats were liable to post-partum hæmorrhage and their young to die at birth or during the nursing period, even if abortion were escaped. These neonatal deaths were characterised by hæmorrhages varying in volume and location, gastric stasis, emaciation, and myelin degeneration of the vagus and phrenic nerves.

Moore and Brodie consider that this hæmorrhagic tendency is due solely to deficiency in vitamin B, and that lack of vitamin C plays no part in its production.

Similar lesions have been observed by Sure and Schilling in young rats. At autopsy hæmorrhages in the osteogenetic tissues were constantly seen, the most common site being along the junctions of the cranial and facial bones and in the tibia and femur, but it was seen quite often in all the other bones examined. Histological examination showed the red areas to be true hæmorrhages. Petechial hæmorrhages were found in the subcutis and internal organs in the failing and dying young, but with far less regularity than the bone lesions, the kidney being the commonest site. Section showed masses of red corpuscles lying outside the vessel wall.

Anhydræmia and marked disturbance in hæmatopoietic function in the nursing young of the rat suffering from uncomplicated vitamin B deficiency are also reported by Sure and Smith (1929).

In the human newborn also cases of hæmorrhage have been recorded.

In a case reported by Moore and Brodie poverty had led the expectant mother to adopt a diet markedly deficient in vitamin B. At the confinement profuse hæmorrhage occurred, and the child, though apparently healthy at birth, developed rapid pulse and respiration, hæmaturia, jaundice, and diarrhœa, resulting in death.

The post-mortem revealed hæmorrhage from the right suprarenal and kidney, retro-peritoneal hæmorrhage, cortical and medullary hæmorrhage from the left kidney, together with myelin degeneration of certain nerves.

Vogt (1929) has also called attention to the danger that deficiency of vitamin B in the maternal diet may lead to abortion or premature death of the child.

(O) **CAPACITY FOR MUSCULAR WORK.**—A somewhat incomplete observation of Csik and Bencsik (1927) seems to indicate that diets poor in vitamin B reduce the capacity for muscular work. This capacity was tested by the use of certain types of ergographs and dynamometers, and though indications were obtained that the performance of work was rather better during the periods of vitamin B feeding, many of the results could not be interpreted owing to difficulty in standardising the conditions of the experiments.

(P) **"AUTOMATINE" AND VITAMIN B₁.**—Substances which have an excitant action upon living tissue have been called "automatines" and, in the case of cardiac tissue, "heart hormones." An ultrafiltrable, thermostable substance has been found in the hearts of the eel and frog, which has been named "automatine," and is probably identical with other active substances found by other workers and named cardiac hormones, etc. "Automatine" (more exactly two automatines X and B) appears to be formed from a mother substance ("automatinogène") which is found most abundantly in muscle. This mother substance can be activated *in vivo* or *in vitro* by radiation with soft B rays for a few hours. The heart of an eel has thus been kept beating in artificial media deprived of potassium for more than 10 hours.

Rigler (1928) found that vitamin B, isolated as a pure chemical substance by Jansen and Donath, belongs to this group of substances. He believed it to be identical with histamine.

Zwaardemaker (1928), however, has carried out experiments to show that although this substance in itself is without "automatin" action it acquires this property through radiation. A specimen of Jansen and Donath's pure preparation was subjected to radioactive rays for a minimum of 4 hours, and in every case in repeated trials, the radiation conferred upon it the properties of "automatine."

In correlating this property of vitamin B₁ with the condition of the heart in beri-beri, Wenckebach (1928) points out that not every heart that stops its systolic action has lost its regular automatic vital function. The automatism may go on undisturbed, but is prevented from manifesting itself by lack of conductivity or, as in beri-beri, by interference with contractility. He thinks that in such cases the active substance which restores the regular function should not be called an automatin; what happens in the heart is not revival from death, but release from bondage.

(Q) **PHARMACOLOGICAL ACTION OF VITAMIN B₁.**—The pharmacological behaviour of vitamin B₁ has been investigated by several workers, but unfortunately their conclusions are not in entire agreement. Uhlman (1918) found effects similar to those of pilocarpine. Voegtlin and Myers (1919) considered that extracts of vitamin B had a secretin-like action on the pancreas, but their findings were not confirmed by Anrep and Drummond (1921) or Cowgill (1921).

Experiments with orypan (an extract from oats, rice, or wheat), which is essentially an extract of the antineuritic vitamin were stated by Burgi (1921) to prove a strong pharmacological effect of vitamin B₁. These extracts exercised a stimulating effect upon the parasympathetic nervous system, upon muscle, upon the motor, and perhaps also the sensory nerves, and chiefly upon the respiratory centre. Burgi also stated that paralysis of the motor centre by morphine could be entirely eliminated with orypan administration while the narcotic

effect remained, and he suggested that the lower toxicity of opium might be explained as due to its content of antineuritic vitamin. Leszcynski (1926) also claimed to have found a parasympathetically active substance in yeast fractions which remains after heating to 150° C.—a procedure which destroys the vitamin. It has been suggested that the presence of histamine and choline in extracts is likely to explain these effects, but in Burgi's experiments the possible interference of choline at any rate was claimed to have been eliminated.

Stimulation of the vegetative nervous system by vitamin B₁ is to be presumed from the hypothesis of Shiroki (1929) that beri-beri is due to a "neurasthenia sympathica." On this hypothesis—that lack of vitamin B₁ leads to inanition of the sympathetic nervous system—Shiroki explains the interaction of all the secondary factors, such as climate, season, sex, occupation, psychic disturbance, which have been shown to exert an influence on the occurrence of beri-beri. All these factors, he points out, involve an excessive stimulation of the sympathetic, followed eventually by some degree of atrophy, thus increasing the malnutritive effect of vitamin B₁ deficiency.

The recent reinvestigations of Peters (1930) on the pharmacological effect of vitamin B, as represented in purified extracts of torulin, have, however, had negative results. They failed to obtain responses with doses of from 10 to 40 pigeon units of torulin upon the virgin guinea-pig's uterus, the frog's heart, the rabbit's heart, and upon an anæsthetised pigeon preparation. They conclude that torulin cannot be considered to have a pharmacological action in the ordinary sense.

(R) **HYPERVITAMINOSIS B₁**.—The evidence for injurious effects of an excess of vitamin B is not very convincing.

A condition related to status thymicolymphaticus has been stated by Scheer (1925–1926) to occur in rats fed on a ration consisting of a yeast product and milk *ad lib.*, and therefore supposed to contain an excessive amount of vitamin B. A comparison of the weights of the organs of these rats compared with those fed on a mixed control diet showed increased weight of thymus, hypoplasia of adrenals, and general lymphatic hypertrophy, and Scheer regards these results, the opposite of conditions prevailing in vitamin B deficiency, as indicative of a true hypervitaminosis.

The suggested association between malignant disease and B hypervitaminosis has been investigated by Jackson and Krautz (1929). They failed to find any evidence that malignant tissue contains unusually large amounts of vitamin B. In fact, it is inferior in this respect to normal liver. They consider that their observations do not favour the hypothesis that malignant disease is due to, or associated with, excess of vitamin B.

The basal metabolism is apparently unaffected by excessive amounts of vitamin B. Mitchell and Carman (1926) determined the basal metabolism by the Haldane method, the respiratory quotient from O₂ consumption and CO₂ production, and the rate of heat production in rats on a constant ration plus yeast. The respiratory quotient of the fasted rats was nearly constant at 0.75. For rats 90 to 190 days old the basal metabolism when referred to body surface (but not when referred to weight) was greater and more variable for the male than for the female; the average found in calories per square m. for the male was 600 ± 11 and for the female 571 ± 7. For both sexes the variability was higher on the weight basis than on the surface basis. Within the above age limits age did not affect basal heat production, but that of younger rats was higher. The ingestion of excessive amounts of vitamin B up to 10 times the estimated requirement did not modify basal heat production.

(S) **REFECTION**.—Fridericia in 1925 observed a spontaneous cure in one of 3 young rats being fed on a vitamin B-deficient diet containing 57 per cent. of rice starch. In the third week, when weakness of the hind legs had appeared, the rat suddenly started growing at a normal rate whilst its faeces became white and bulky. It was then found that the effect

could be transmitted to other young rats, on a similar diet, by adding these white bulky faeces to the diet.

This phenomenon, called "refection" (Latin: *reficere* = restore), was also observed to occur spontaneously in rats at the Lister Institute in September 1927. Rats become refected after a few days' feeding with faeces from refected rats, and this state may then exist for months or years, the rats growing normally, mating and producing offspring with other refected rats, all being reared on a vitamin B-free diet.

Fridericia was unable to produce refection in rats receiving adequate vitamin B by faecal feeding, but Roscoe (1927) noted the appearance of white, bulky faeces not only in rats deprived of vitamin B, but in some receiving the vitamin or one of its two known components. After refection was established in rats receiving vitamin B they continued to grow when the doses of the vitamin were stopped. In one case a spontaneously refected rat was placed in a cage the wider mesh of which allowed the large faeces to fall through with greater ease than usual. The rat's faeces became dark, and after 3 weeks the animal died with paralysis. Other rats in similar cages declined at first but subsequently recovered. It was inferred that to continue in the refected state it was essential for the rats to eat some of their own faeces, for some rats consume their faeces as they leave the body. Refection was then attempted by feeding the faeces of refected rats to other rats, and success was attained in most cases even when one or both of the water-soluble vitamins B was added to the basal diet.

The faeces of refected rats contain a large percentage of starch (59 to 62 per cent. in Fridericia's cases). The white faeces contain more amylase than the brown faeces of non-refected rats, the amylase being able to dissolve grains of rice starch but almost unable to act on the starch grains of the white faeces. Fridericia believed this resistance of the starch to amylase to be due to some substance adsorbed upon the grains, a substance which is soluble in acid alcohol. He stated that the faeces of refected rats contain considerable amounts of vitamin B after the refecting virus has been destroyed by heat, though the brown faeces from non-refected rats on a vitamin B-free diet contain very little.

Roscoe's experiments, however, suggested that refected faeces contained very little of the antineuritic vitamin. The presence of a large proportion of rice starch in the diet was found to be essential for the induction of refection. Fridericia and his colleagues found refection impossible when the starch of the experimental diet was replaced by sugar, but possible when it was replaced by dextrin containing a small proportion of starch. The results of Roscoe's experiments agree in general with these conclusions. One rat received all its carbohydrate in the form of corn dextrin. Its previous condition of refection disappeared, the faeces became small and dark, and paralysis occurred, though 1.0 gram of dried faeces from refected rats was administered. The paralysis was cured by feeding yeast extract containing only the antineuritic factor, and after this the rat grew normally as long as the faecal dose was given, in spite of the lack of this principle.

Replacement of the rice starch by potato starch was found by Kon and Watchorn (1928) to induce refection with considerable regularity. Raw arrowroot starch induced some degree of refection, the animals fed on the latter thriving much better than those on raw rice starch, but less well than those on raw potato starch. Their results, however, have not been confirmed by Mendel and Vickery (1929), who were unable to induce refection by either commercial corn starch or potato starch. Experiments were also undertaken to determine whether refection would be possible if the starch was altered by cooking. Refection ceased when refected animals were fed on a cooked rice diet, and definite cases did not occur spontaneously. Kon and Watchorn (1928) also found that the ability of potato starch to induce refection is diminished by extraction with 70 per cent. alcohol and is largely destroyed by gentle cooking of the starch.

In the experiments of Taylor and Thant (1929) on pigeons the starch of dhal was found to be favourable to the occurrence of refection. When the dhal was cooked instead of autoclaved the occurrence of refection was more marked. They suggest that there may be two factors concerned in this definite association of cooked dhal with refection: (a) a suitability of the cooked dhal for its production; (b) the influence of a degree of vitamin B deficiency rather than its complete absence, the process of cooking used not causing complete destruction of the antineuritic factor.

(1) **Explanation of Refection.**—The explanation of refection adopted by Fridericia is the presence in the alimentary tract of the refected animal of some virus which produces the material necessary to correct the defective diet. He states that the fæces and the cæcal contents contain the virus of refection and can be kept in a dry state for $5\frac{1}{2}$ months without loss of power to reffect. The virus is killed by heating at 100° C. for 10 minutes, but not by heating at 80° C. for 10 minutes. Many abnormal varieties of organisms are found in the fæces, but attempts to isolate the virus have been unsuccessful.

(2) **Intestinal pH in Refection.**—In non-refected rats on a vitamin B-deficient diet the cæcal contents are alkaline; in refected rats they are acid (Fridericia *et al.* (1927), Schoubye (1928)).

(3) **Importance of Refection.**—It has been pointed out by Fridericia that the occurrence of refection must be borne in mind in all statements of the minimum vitamin B requirements of different species. When refection is present this minimum may be zero.

(T) **MALIGNANT DISEASE.**—The evidence for the connection between vitamin B deficiency and the development of malignant tumours is conflicting, but the final result appears to be that avitaminosis B does not in itself influence the growth of tumour tissue. The majority of workers, including Benedict and Rahe (1917), Sugiura and Benedict (1920), have reported negative results; while others, including Taniguchi (1928) and Heyman and Gallinek (1928), have observed a decrease in the growth of transplanted sarcoma when the diet is deficient in vitamin B. In Taniguchi's experiments transplanted sarcoma showed the least growth in hens on a vitamin B-deficient diet and grew outward as a closed mass, while on the vitamin B-rich diet the sarcoma penetrated irregularly into the tissue. There was a distinct difference in the histological picture in the cells from both groups after 6 days.

In Heyman and Gallinek's observations of young black-and-white rats injected subcutaneously with Jensen sarcoma, 56 per cent. gave positive "takes" on a vitamin B-free diet, while of those on a normal diet 80 per cent. developed new growths. In the animals on a normal diet the tumours reached 77 per cent. of the body weight in 30 days, while in only 9 per cent. of those on the vitamin B-free diet did the tumours reach over 10 per cent. of the body weight. In the normally nourished rats the average relative tumour weight was 3.8 times as great as in those on a vitamin B-free diet.

These observations do not agree with those of Solimano (1926), who stated that a polished rice diet appeared to favour the development of inoculated sarcoma in rats. A series fed on polished rice before inoculation developed tumours 21 days (average) before controls fed on a liberal diet. The duration of life was decreased 50 to 90 days. The animals fed polished rice from the time of inoculation developed tumours 9 days (average) before the controls and died 5 to 27 days earlier.

The explanation put forward by Drummond (1917) perhaps best reconciles the various findings. Drummond found that when certain restrictions of diet were imposed, such as a ration poor in protein, containing protein of poor nutritive value, deficient in certain amino-acids, or deficient in vitamin B, the growth of tumours remained uninfluenced so long as the host was able to supply the missing elements from its own tissues. When the host is unable to make good the deficiency by drawing upon its own reserves, the rate of tumour proliferation

will decrease. This occurs at a comparatively early stage when the diet is deficient either in tryptophane or vitamin B.

It should be noted that a somewhat tentative conclusion has been reached by Nakahara and Somekawa (1929) to the effect that the reserve of vitamin B in the body is not depleted by the growth of Rous chicken sarcoma. The vitamin B reserve in the liver of tumour-bearing and healthy chickens was found to be essentially the same. Nakahara (1929) also points out that experiments indicate that the Rous chicken sarcoma is habitually deficient in its vitamin B content. The daily feeding of as much as 1 gram of dried sarcoma tissue afforded absolutely no protection against the development of polyneuritis in pigeons, nor did 1 gram daily of dried sarcoma show any sign of preventing the decline in body weight of the rat on a vitamin B-deficient diet. Flexner-Jobling rat carcinoma and Fujinawa rat sarcoma, on the contrary, were found to contain vitamin B, but in relatively small amounts. He concludes that while vitamin B synthesis by tumour tissue seems highly improbable, that possibility cannot be absolutely excluded. There may exist some qualitative difference in the nutritional requirements between normal and malignant tissue growths.

LIII. VITAMIN B₁ IN FOODSTUFFS.

Vitamin B₁ has a very wide distribution amongst natural foodstuffs. The germ of cereals and yeast are its richest sources. Egg-yolk, fresh vegetables, fruit, fresh meat, and milk contain it also, but in much smaller amount.

Since the division of the vitamin B complex into various factors, and the recognition that while one fraction is required by pigeons and not by rats, another is required by rats and not by pigeons, much of the testing of foodstuffs for vitamin B is merely an indication of their content of one or more factors of the complex. Most of the values recorded hitherto have shown the amounts of food considered as a source of vitamin B necessary to rat growth or to pigeon health, but have not differentiated between their antineuritic and antipellagric content.

The heat labile fraction prepared by Williams (1928) by refinements in the control of the adsorbing power of fuller's earth produces in rats on a diet otherwise deficient in vitamin B a slight amount of growth. Autoclaved yeast alone also stimulates growth at first, but is soon followed by death from polyneuritis. By combining Williams' preparation with autoclaved yeast normal rat growth is obtained and polyneuritis prevented in both rats and pigeons. With the aid of these two substances—Williams' preparation and autoclaved yeast—it is possible to differentiate the two factors in any given foodstuff. As an example of this method the estimation of vitamin B₁ and B₂ in banana by Eddy (1928) may be quoted. He found that if the antineuritic factor were supplied by a source such as Williams' preparation, which is at the same time free from the B₂ factor, even 2 grms. of banana per rat per day supplied enough of the B₂ factor to produce fairly good growth, while 6 grms. of banana produced enough B₂ for nearly normal growth. The banana is then a good source of B₂. On the other hand, if B₂ were supplied by autoclaved yeast devoid of B₁, rats failed to show good growth until at least 6 grms. a day was added to supply the antineuritic factor. The banana, therefore, seems to be a better source of vitamin B₂ than B₁. Other foodstuffs will no doubt in time be thus differentiated, but for the present, unless specifically stated, the vitamin B₁ content as described below will be assumed to include the other factors of the vitamin B complex.

(A) **CEREALS**—(1) **Wheat**.—The prominent place occupied by wheat in the diet of mankind was, until the discovery of vitamins, attributed to its content of protein superior in nutritive value to the proteins of other cereals. It was only after the investigation of the wheat grain for its vitamin content that it was realised that wheat retained its efficacy in nutrition only if certain portions of it were left intact.

Osborne and Mendel (1919) and Bell and Mendel (1922) found that the vitamin B is

present in the embryo, the endosperm, and the pericarp, the embryo containing actually about one-sixth of the total amount in the grain. From this fact has arisen what is known as "The Brown and White Bread Controversy." The protagonists of brown bread maintain that brown bread is better than white because it contains more protein of a higher biological value, more vitamin B, more mineral matter, and more roughage. The white bread supporters maintain that an ordinary mixed diet contains enough of these elements to compensate for their absence from white bread, and further, that the yeast used in baking provides sufficient amount of B₁. The question is further complicated by the realisation that in most of the experiments performed the good or bad effects observed were partly due to the presence of the growth-promoting as well as the antineuritic factor of vitamin B in whole wheat.

The process of milling, with the introduction of fine wheat flour for the baking of bread, has changed considerably the outlook on bread as a vitamin-containing article of diet.

The following account of the process is taken from the data given by Cramer and Mottram (1927):

"The wheat grain consists of the endosperm which makes up the bulk of the grain, about 77 per cent., the embryo, which constitutes only 2 per cent. of the grain (and is richer in fat than the endosperm), and the husk. The latter is made up of two layers, which are fused together—namely, the pericarp or modified ovary wall, and the testa or seed coat. Immediately underneath the testa or seed coat is a layer of differentiated cells, the aleurone cells. In the process of milling the grain is disrupted and a number of materials are obtained from it, which are separated from each other by the size of the sieve through which they are made to pass. In this manner the particles which go through the finest mesh constitute white flour, of which about 70 per cent. of the whole grain is obtained. The next coarser material is called 'sharps' or 'middlings,' and in the main this is made up of the inner skin of the grain together with the aleurone cells, with a slight admixture of endosperm and also of embryo. The coarsest material is the 'bran,' which is essentially the outer skin, again with a slight admixture of the wheat embryo. The bulk of the embryo is separated from the other materials owing to its fatty nature. This product, in which a considerable amount of endosperm remains adherent to the embryo, is called technically the 'germ.' Thus as a result of milling one obtains the different materials in the following proportions:

	Per cent.			
White flour	70
Sharps or middlings	14
Bran	14
Germ	1½–2
Loss	½

"All the milling products except white flour are sold as offal and used for cattle food, except where wholemeal or germ flour is being used for special kinds of bread. The white flour may again be graded in the process of milling according to the requirements of the trade. The finest white flour is called 'Patent.' This represents 60 to 75 per cent. of the total white flour extracted in milling. 'Straight run flour' is simply the total ungraded white flour as obtained in the milling."

The richness of the embryo in vitamin B₁ was emphasised by McCollum and co-workers (1915 and 1916), and has been confirmed by other workers, notably by Morgan and Barry (1930). Further investigations of the various products of milling were made by Chick and Hume (1917, 1919) testing them with regard to avian polyneuritis. They found wheat germ to be richest in vitamin B; next came bran, while white flour was so poor in vitamin B that by itself it was unable even to protect birds against polyneuritis or man against beri-beri. The antineuritic potency of wheat germ was found to be very high, a dose of 2.5 grms. or less being sufficient to cure a pigeon showing fully developed symptoms.

In the experiments of Osborne and Mendel (1916) and Bell and Mendel (1922) also the germ itself was found to be the richest in vitamin B₁, while the other products examined, "first and second clear," "low grade," and "bran," and standard middlings were progressively higher in vitamin B content. A more detailed description of the milling products was given by Cramer and Mottram (1927).

They took as their indicator of the absence of vitamin B the lowering of the body temperature in rats kept in a room at a warm and even temperature. The animals were kept on the basal vitamin B-free ration until the body temperature had fallen to about 36° C. Then the material to be tested was added to the basal ration. If the temperature returned to the normal in 2 weeks, and the weight increased, the result is stated as "complete recovery." If the normal temperature was regained after a lapse of more than 2 weeks the result is stated as "slow recovery." If the normal temperature was not regained the result is "no recovery." Wheat germ was found to be very rich in vitamin B and at least equal to yeast in that respect. Taking the vitamin B content of yeast as 100, the various milling products of wheat grain are: germ, 100; middlings, 50; bran, 33; patent flour, 0.

The amount of yeast added to flour in baking is at the most 2 per cent. Hartwell's (1924) observations suggested that white bread owed its vitamin B content, which she considered adequate, to the amount of yeast added to it. Cramer and Mottram showed that this amount is insignificant. This was confirmed experimentally, for whilst the addition of 2 grms. air-dried Hovis germ bread (made from flour containing 1 part of sterilised germ and 3 parts of patent flour), or 3.5 to 4 grms. wholemeal bread, brought about recovery in vitamin B-deficient rats, the addition of 5 grms. air-dried white bread (50 per cent. of basal ration) or the substitution of bread for casein (100 per cent. of basal ration) was unable to affect recovery and the rats died.

Experiments on the addition of yeast to white bread in sufficient quantity to give the bread an adequate vitamin B content have been made by Veselkin and co-workers (1927). They fed pigeons on white bread containing varying amounts of bakers' yeast and with additions of brewers' yeast dried at 40° C. It was found that 0.2 gram of dried brewers' yeast fed daily separately with white bread *ad lib.* provided enough vitamin B to maintain weight and to prevent polyneuritis. Since 0.5 gram brewers' yeast was needed to provide sufficient vitamin B with a vitamin B-free basal diet, it was concluded that white bread containing 0.5 per cent. bakers' yeast provided about half the vitamin B necessary to maintain weight. Attempts to increase this vitamin content of bread by additions of bakers' yeast up to 6 per cent. of the total weight were unsuccessful, but additions of 2 per cent. dried brewers' yeast to the dough containing 0.0 per cent. bakers' yeast produced a bread on which the pigeons maintained their weights constant.

It was concluded that it is possible to prepare a white bread containing sufficient vitamin B without changing its taste, by the addition of dried brewers' yeast.

The same conclusion has been reached by Willimott and Wokes (1928). From their experiments with dried yeast they found that the minimum curative dose for rats was 0.3 to 0.5 gram daily, from which they calculated that it would be necessary to add 3 to 4 per cent. of dried yeast to white flour in order to ensure an adequate amount of vitamin B in bread for human consumption. The diastatic enzymes of the yeast could first be destroyed by gentle heating for a short time. They claim that the addition of 4 to 5 per cent. of a suitable dried yeast gives no disagreeable flavour to the bread.

Scheunert and Schieblich (1928), however, state that the amount of yeast necessary to give an adequate vitamin B content to white bread would be so large that the result would no longer be a wheat bread in the ordinary sense, but a yeast bread.

Cramer and Mottram suggest that the main supply of vitamin B in the diet used by

Hartwell came from the butter which she incorporated, and not from the white bread. The final outcome of the discussion, so far as vitamin B₁ is concerned, in spite of the later observations of Hartwell and Mottram (1929) to the effect that propaganda for brown bread in the working classes is unnecessary, appears to be that white bread is distinctly deficient in antineuritic value as compared with brown bread. This does not mean, however, as pointed out by Cramer and Mottram, that white bread must be replaced by wholemeal as a universal article of diet. In fact, since flour containing any part of the germ does not keep well, it would be practically impossible to adapt it for the supply of countries with dense populations. Any desired mixture of the milling products which contain vitamin B₁ can be added to the white flour, and these, having been separated, can be treated by methods which retard its deterioration without destroying its vitamin content.

The vitamin value of whole wheat has been found by McCarrison (1927) to be very high, 1 gram of wheat containing as much vitamin B as 0.35 gram of marmite.

(a) INFLUENCE OF MANURIAL CONDITIONS ON THE VITAMIN B CONTENT OF WHEAT.—McCarrison and Viswanath (1926) and Hunt (1927) have investigated the relation between the vitamin B content of wheat and the soil in which it is grown. McCarrison found that cattle manure gave a wheat of vitamin value about 15 per cent. higher than chemical fertiliser, but Hunt's experiments indicated that acid phosphate, alone, or in a complete fertiliser with KCl and NaNO₃, produces wheat with the highest vitamin B content.

(b) WHEAT GERM SUGAR.—A preparation of dextrin and maltose prepared from the embryo of wheat has been stated by Dennett (1929) to be very rich in vitamin B₁. He has suggested that this preparation should be added to cows' milk for infant feeding.

(2) Rice.—As in the case of wheat, the richest source of the antineuritic vitamin is the germ, which is removed with the bran during steam milling. McCarrison (1927) found the vitamin value of rice considerably below that of wheat, but Kintaro (1927) has stated that a quantitative analysis shows wheat and rice embryo to contain approximately equal amounts of vitamin B. A dosage of 0.5 to 1 gram of rice germ, in terms of the natural foodstuff, picked out from the unmilled grain by hand, or 10 grms. of rice bran containing germ, has been found by Chick and Hume (1917) to effect a complete cure of polyneuritis in a pigeon 300 to 400 grms. in weight.

(a) INFLUENCE OF AGE AND MILLING PROCESSES ON THE VITAMIN B₁ VALUE OF RICE.—A study of the preventive action of rice in various stages of milling has been made by Kessler (1927). He found that uncured and unhusked rice maintained pigeons in perfect health, as did also undermilled rice, still in its husk, even when 3 years old. Husked and steamed rice and polished rice produced symptoms within 70 days, but health was restored by the administration of bran.

(b) PARCHED RICE is stated by Mathur (1930) to retain vitamin B₁. Pigeons fed on parched rice (bought in the open market) showed no symptoms of polyneuritis after 46 days, while control birds fed on polished rice showed definite signs of polyneuritis in the third week.

(c) INFLUENCE OF STORAGE.—Rice apparently undergoes little deterioration if it is kept unhusked and not too hot (Kessler 1927). According to Kondo and co-workers (1929) rice kept for 4 years in impervious vessels containing CO₂ or air had as great a vitamin B content as freshly harvested rice. Undermilled rice, however, in Kessler's experiments, if not quite fresh, lost its vitamin content after some months.

(3) Maize.—The preparation of maize has been shown by Jansen and Donath (1928) to determine its vitamin B₁ content to a large extent. If the germ and pericarp are removed with the aleuron layer, the maize may be entirely devoid of the antineuritic vitamin. The best method of preparation was found to be that of Madura, which results finally in almost the whole of the grain being eaten.

Chick and Hume (1917) have found that 1 to 3 grms. of maize germ (in terms of the natural foodstuff) cure polyneuritis in a 300 to 400 gm. pigeon.

(4) **Rye, Barley, Oats, etc.**—(a) RYE EMBRYO has been stated by Scheunert (1927) to contain slightly more vitamin B than wheat.

Barley in quantities of 3.7 grms. unhusked and 5 grms. husked has been found by Cooper (1912, 1914) to afford protection against polyneuritis in pigeons 300 to 400 grms.

(b) GERMINATED BARLEY, according to Schittenhelm and Eisler (1928), contains a relatively large amount of vitamin B, 3 grms. daily preventing beri-beri and inducing recovery from nervous symptoms.

(c) MILLET, steamed, dried, and roasted to produce "Chao-mi," the chief food of the Mongolians, has been found protective against avitaminosis in pigeons by Abe (1928).

(5) **Other cereals** have been tested by Plimmer and Rosedale (1927) by determining the least quantity on which the birds could be maintained with healthy reproduction over a long period. Using dried yeast as the standard, and taking its value as 100, the following were obtained :

				Per cent.
Dried yeast	100
Bakers' yeast	33-40
Marmite...	40-50
Wheat germ	66
Whole wheat	8-10
Rye	9
Barley	7-8
Oats	4-5
Oatmeal...	4
Buckwheat	5-6
Bran	12-13
Middlings	12-13
Maize	7-8
Millet	8
Dari	8

When oatmeal was the cereal used, the young could not be reared without the addition of marmite to the diet.

Plimmer states that in order to determine the amount of cereal that should be present in a diet to provide sufficient vitamin B for maintenance (not necessarily for rearing), the quantity or quantities that supply enough to give a total of 4 should be estimated. For chickens the amount should be calculated on a basis of 6, and probably in the case of man on a basis of 3. Thus, 20 per cent. of millet gives 1.6, 10 per cent. of dari 0.8, 40 per cent. of whole wheat 3.2—total, 5.6. The remainder of the diet can be white rice, white flour, fish meal, or any other foodstuff without vitamin B.

(B) **PULSES AND NUTS**—(1) **Beans.**—(a) THE KATJANG-IDJO BEAN (*Phaseolus radiatus*) was shown by Grijns (1901) to have distinct value in the prevention and cure of avian polyneuritis and human beri-beri, and his findings have been confirmed by Hulshoff-Pol (1917).

(b) THE MUNG BEAN (*Phaseolus aureus*), which forms an important part of the Chinese dietary, has been found by several observers to contain a moderate amount of vitamin B before germination. Heller (1927) states that when it composes 60 per cent. of the ration its vitamin B content is adequate.

During germination of beans the vitamin B content increases (Kim, 1928; Miller and Hair, 1928). The latter workers have investigated the sprouts produced from the green beans, which constitute a considerable portion of the diet in the oriental population of Honolulu. They concluded that the amount of raw sprouts in the daily ration supplying vitamin B and maintaining weight is between 2.5 and 3 grms., and 2.2 and 2.7 for the cooked. The latter weights are equivalent to those given for the raw, there being little loss of vitamin B in cooking.

Compared with other vegetables on Sherman's basis, bean sprouts show 150 to 180 units per pound as against 150 to 200 for lettuce, 120 to 140 for carrots, and 1000 for raw peas.

(c) **STRING BEANS** are stated by Quinn, Burtis, and Milner (1927) to contain vitamin B in a concentration of about 0.3 units per gram.

Other varieties of beans, *Soya max Linnæus*, and the red and white varieties of *Sorghum vulgare Persoon*, and their sprouts have been tested by Sherman (1929) and found to contain varying amounts of vitamin B.

(2) **Peas and Lentils**.—Dried peas, in a dosage of 40 grms. of the natural foodstuff, and dried lentils in a dosage of 20 grms., were found to cure polyneuritis in pigeons by Cooper (1912, 1914), and these results have been confirmed in rats by McCollum, Simmonds, and Pitz (1917), and Osborne and Mendel (1919).

Various other kinds of lentils, including *Phaseolus mungo*, *Cicer arietinum*, and *Cejanus indicus*, have been tested by Ghose (1922) and found to contain appreciable amounts.

Peas.—Green peas are stated by Eddy and co-workers (1926) to be a rich source of vitamin B, richer than milk, tomatoes, or oranges. Large mature peas contain more than small young ones. Canned peas are as potent as raw, the vitamin B content being unaffected by the process.

Plimmer and co-workers (1927) have worked out the vitamin B value of some pulses and nuts on the basis of yeast=100. The pulses, including split peas, whole dried peas, lentils, haricot beans, and soy beans, were found to have an almost equal value of 13, while nuts were more variable, coconuts being of no value.

	Percentage Amount in Diet for Maintenance.	Relative Vitamin B Value.
Dried yeast	4	100
Split peas	30	13
Whole dried green peas	30	13
Lentils	30	13
Haricot beans	40	10
Soy beans	30	13
Peanuts	20	20
Ground almonds	40	10
Whole almonds	40	10
Hazel nuts	20	20
Dried chestnuts	40	10
Coconut	No maintenance	0
Coffee, green	Over 30	Less than 13
Coffee, roasted	No maintenance	0

(It should be noted that the above vitamin B values are not worked out for vitamin B₁ and B₂ separately.)

(3) **Ground-nut meal** (peanuts), and biscuits made from it, has been tested for its anti-beri-beri value by Greig (1918), and has been found curative in a dosage of 9 grms. per day.

(C) **EGGS**—(1) **Yolk**.—Vitamin B₁ is present in a fairly high concentration in the yolk of eggs. According to Cooper (1912, 1914), 60 grms., equivalent to 4 yolks, is sufficient to cure polyneuritis. A water extract of fresh egg-yolk has been tested by Osborne and Mendel (1923) and found potent in a dosage of 1.5 gram for a 100-grms. rat. On this basis they state that 1 egg is equivalent to 150 c.c. whole milk. Sherman (1927) estimates the vitamin B content of egg-yolk as 50 units per ounce, compared with milk—9 units. Dried whole egg, according

to Chick and Hume (1917), retains its curative activity in a dosage of 40 to 20 grms. (=4-2 yolks), but Osborne and Mendel (1919), found it only half as rich as spinach, and McCollum and Davis (1915) found 5 per cent. of the diet of desiccated egg insufficient to cure polyneuritis.

(2) **White.**—Randoin and Simonnet (1927) state that white of egg is devoid of vitamin B₁.

(D) **YEAST.**—Yeast has been largely used as a source of the antineuritic vitamin in experiments on rats, and in commercial preparations as extracts, tablets, etc.

(1) **Fresh Brewers' Yeast.**—According to Cooper (1912) the activity of fresh brewers' yeast is 60 per cent. of the activity of wheat embryo and slightly more active than dried yeast.

Scheunert and Schieblich (1929) have compared brewers' yeast with two kinds of compressed yeast, one made from molasses and the other from a grain mash. The brewers' yeast was found to be about three times as potent as the compressed yeast. Fresh brewers' yeast is stated by Williams (R. J.) (1921) to be less active than bakers' yeast.

(2) **Yeast Extracts.**—These extracts, of which the best known is marmite, are usually obtained by heating the yeast under proper conditions with strong saline solutions. The saline solutions employed vary in strength, from 2 to 10 per cent. or more, and the time of heating also varies from half an hour to several hours. The destruction of the yeast enzymes is thus ensured, without loss of vitamin potency. The bitter taste of the yeast can be overcome by extracting with a suitable strength of alcohol (75 to 80 per cent.), but on the commercial scale admixture with suitable fat, such as bacon fat or cotton-seed oil, is usually employed.

Plimmer (1928) has tested marmite and also other samples of yeast extract, and has discovered a variation in vitamin B potency of 200 or 300 per cent. Kennedy and Palmer (1922) also found yeast to be very variable in potency.

A yeast extract has been prepared by Willimott and Wokes (1928) from brewers' yeast by extraction with 75 per cent. alcohol, followed by heating to 98° C. with a 5 per cent. saline solution. This extract, though containing only two-thirds the amount of total extractive matter in marmite, was found to be at least as potent as marmite, the minimum adequate dose being about 0.6 gram per day. From these experiments Willimott and Wokes support Plimmer's suggestion that marmite has diminished in vitamin potency of recent years, though they point out that the more accurate methods of testing for vitamin B, with regard to the other constituents of the diet, and also the presence of more than one factor in the vitamin B complex, may account for at least some of the apparent deterioration.

Commercial yeast extracts tested by Chick and Hume (1917) proved protective against polyneuritis in doses of 1 gram per day and curative in doses of 1 to 5 grms.

(3) **Dried Yeast.**—Experiments by the Pharmaceutical Society of Great Britain (1927) have shown that the removal of moisture from yeast yields a product of a potency of 0.3 to 0.5 gram. This is equivalent to 3 to 5 per cent. of the diet, some unusually potent dried yeasts being effective in the proportion of 2 per cent. In the experiments of Willimott and Wokes (1928) a dried yeast was prepared from the residue from which the alcoholic extract had been made. The residual sludge was dried by passage over steam-heated rollers in such a thin layer that less than 1 minute's exposure to the temperature of 100° C. sufficed to drive off practically the whole of the moisture. By this means any destruction of vitamin B was avoided. The product was in the form of thin light brown flakes, which were readily reduced to powder. Less than 1 per cent. of this powder failed to pass through a sieve of No. 40 mesh. Thorough mixing of the sieved powder ensured a product of uniform consistency, truly representative of the residue from which it had been obtained. The powder, when stored without any special precautions, underwent no noticeable deterioration in 12 months. Tests showed it to be free from diastatic enzymes. It was then fed to 3 rats suffering from vitamin B deficiency in doses of 0.25, 0.5, and 1.0 gram respectively.

The results indicated that 0.5 gram produced rather better growth response than 0.25

gram of marmite ; 1.0 gram was about equal to 0.5 gram of the experimental extract. Allowing for the water content of the latter, the actual ratio of curative doses in the solid matter was therefore about 4 in the extract to 1 in the residue. Hence it would appear that the method of extraction employed had taken out roughly four-fifths of the total amount of vitamin B present in the raw material.

On account of the gastro-intestinal disturbances sometimes experienced after a dosage of more than 10 to 20 grms. a day, Schittenhelm and co-workers (1927) have attempted to obtain a palatable and well-tolerated preparation. These preparations—(1) dried yeast, from which the greater part of the nuclear material had been removed ; (2) the same yeast baked at a high temperature ; (3) a mixture of malt and the baked yeast—were found satisfactory. The following table, taken from Willimott and Wokes' paper in *The Lancet* (1928), shows the potency of the different forms of yeast :

VITAMIN B CONTENT (COMPOSITE FACTORS) OF DRIED YEAST AND YEAST PRODUCTS.

	Moist Yeast. ¹	Dried Yeast.		Yeast Extracts.	
		From Marmite. ¹	From Experimental Extraction.	Marmite. ⁵	Experimental Extraction.
Minimum curative rat dose (gram) ²	0.5–0.8	0.2	1.0	0.4–0.6	0.6
Minimum percentage in diet of rats ³	5–8	2	10	4–6	6
Minimum percentage in human diet ⁴	7–12	3	15	6–8	9

¹ Data given by Plimmer and other workers.

² Minimum dose which will restore normal growth when supplied as sole source of vitamin B to standard rats of 40 to 60 grms. weight suffering from vitamin B deficiency.

³ Assuming that food intake is about 10 grms. per head per day.

⁴ Using Plimmer's figure for vitamin B requirement of human beings, i.e. 1½ times that of the percentage requirement of rats.

⁵ Average results obtained by Bacharach and Hartwell (1927) ; Hartwell (1925).

(E) MILK.—The vitamin B content of milk has been the subject of much discussion, partly because its content is variable, and partly because experimental results have been found to disagree. Even such early workers as Hopkins (1906–1912) and Osborne and Mendel (1911, 1912, 1913, 1915, 1918, 1920, 1922) found the quantity of milk sufficient for satisfactory growth to vary from 2 to 3 c.c. per day to 10 to 16 c.c. It is possible that this particular variability arose from the fact that the basal diets were not sufficiently purified, but in later disagreements other factors have been taken into account, such as the separation of the vitamin B complex into its component parts, the formation of vitamin B by bacterial fermentation in the cow, etc.

The comparative unreliability of breast milk as an optimal supply of vitamin B₁ for the infant has been pointed out by Hoobler Macy and co-workers (1926). Their observations showed that a child fed on the breast may be receiving subminimal amounts of the antineuritic factor. Macy and co-workers (1927) followed up these observations by a quantitative study of breast milk pooled from wet nurses on a normal diet and found it markedly deficient in vitamin B. In another experiment these workers showed that 20 c.c. of cows' milk given as the sole supply of vitamin B was insufficient to maintain growth and suggested that every child should have a separate source of vitamin B added to its milk diet.

Cows' milk sold in Berlin is, according to Reyher (1928), noticeably poor in vitamin B₁.

According to Sherman's (1927) quantitative estimate of the vitamin B value of milk, as compared with that of other foods used in infancy, the value is 9 units per ounce as compared with 50 for egg-yolk, 9 for orange juice, 20 to 24 for spinach, and 8 for potatoes.

(1) **Effect of Maternal Diet on Vitamin B₁ Content of Milk.**—The investigations of Hughes, Fitch, and Cave (1921), and of Kennedy and Dutcher (1922), seemed to show that cows on a deficient diet could not supply milk adequate in vitamin B content. Later experiments, however, notably those of Bechdel, Honeywell, and co-workers (1927), have indicated that the vitamin B content of the milk is not affected by the amount of this factor in the cows' diet. When milk from 3 cows, which had been reared for 2 years on a vitamin B-deficient diet, was fed to growing rats as the sole source of vitamin B, the vitamin B potency was found equal to that of herd milk from cows receiving a good winter ration. All available evidence tends to show that cattle, and possibly all ruminants, differ from other animals in their ability to grow to maturity, produce normal offspring, and to maintain vitamin B in their milk whilst being fed on rations deficient in vitamin B. Further experiments by the above workers indicate a synthesis of vitamin B in the rumen of the cow as the result of specific bacterial activity.

(2) **Concentration of Vitamin B₁ in Milk.**—A concentrate of the water-soluble fraction of milk has been prepared by Supplee, Dow, and Flanigan (1928) by successive removal of the fat or cream by skimming, the casein by heating with a suitable precipitant, and the lactalbumin by coagulating with heat. A considerable portion of the insoluble calcium phosphate was removed, and, by repeated crystallisations, the greater part of the milk sugar. The residual liquor was then concentrated. The concentrate was fed to rats on a basal diet of purified casein, salt mixture, agar-agar, butter-fat, and dextrin. The results showed that the solids of the water-soluble fraction of milk are highly potent in vitamin B content and compare favourably with dried yeast as a source of the growth-promoting and antineuritic factor.

(3) **Dried and Condensed Milks.**—Although temperature and aeration are important factors in the destruction of the antineuritic vitamin, the most usual conditions employed in the evaporation of milk are not such as to destroy its content of vitamin B₁. Dutcher and co-workers (1926) state that only under unusual conditions can vitamin B destruction in evaporated milks be attributed to methods of manufacture.

Drying by the roller process, in which the temperature is not raised above 98° C., appears to be the most satisfactory process. It was shown by Johnson and Hooper (1921) that this process had no effect on the antineuritic vitamin, and by Hartwell (1925) that dried milk made by the roller process was superior in vitamin B content to pasteurised and evaporated milk. Recent experiments by Daniels, Giddings, and Jordan (1929) show that, while some destruction of vitamin B₁ occurred in superheated milk made by the spray process and in desiccated albumen milk, none took place in milk made by the roller process. According to Donath (1929), however, dried and tinned milks used in the Dutch East Indies have suffered a considerable loss of the antineuritic vitamin.

(4) **Pasteurised Milk.**—According to Daniels and co-workers (1929), milk pasteurised by the open method loses its vitamin B₁ content, while that pasteurised by the closed method shows no vitamin destruction.

(5) **Boiled Milk.**—The above workers have also shown that milk heated to boiling temperature quickly and cooled slowly is severely affected, while the same milk cooled quickly shows no adverse effect. These results indicate that temperature and aeration are important factors in the destruction of the antineuritic vitamin in milk.

(6) **Malted milk** is stated by Quinn and Brabec (1930) to be rich in vitamin B₁.

(F) **MEAT.**—Meat, and indeed all animal tissues, are on the whole deficient in vitamin B₁ (Drummond (1919)).

In preventing polyneuritis, Cooper (1912, 1914) found 20 grms. of beef muscle per day necessary, while 140 grms. of raw beef must be given to effect a cure. Beef heart, liver, and brain were found more efficacious both by Cooper and by Osborne and Mendel (1919). Dried beef was found by Hoagland (1929) to contain less antineuritic vitamin than lean pork, a proportion corresponding to a daily intake of from 7 to 8 grms. being necessary, while 5 per cent. of dried lean pork in a ration protected pigeons against both polyneuritis and loss in weight for 8 weeks and longer. This proportion corresponds to a daily intake of 1 gram of dried pork for a 400 grms. pigeon. Fresh and smoked ham were equally good sources of the antineuritic vitamin.

Tinned roast beef, examined by Chick and Hume (1916), proved very deficient in vitamin B₁, 350 grms. daily being insufficient to complete the cure of polyneuritis.

Commercial meat extract, as tested by Chick and Hume (1917), proved devoid of vitamin B₁, and the insoluble residue obtained in its manufacture, even when constituting 40 per cent. of a food mixture having a caloric value of approximately 5.2 calories per gram, was found by Cowgill (1927) markedly deficient.

(G) **FISH** tested by Cooper (1912) gave no protection against polyneuritis even when administered in amounts of more than 10 grms. daily. Whole herring, however, contains some vitamin B, whereas protein-free extracts of the muscle do not; the content probably lies in the ova and other internal secretory glands, which have been shown to be not entirely deficient by Chick and Hume (1917). Eddy (1921) and also Hartwell (1922) state that cod and herring both contain a fair amount.

(H) **VEGETABLES AND FRUITS**.—Of the many vegetables tested, spinach, tomatoes, asparagus, celery, lettuce, and cabbage appear to be the richest in vitamin B₁.

It should be noted that, as pointed out by Scheunert and Schieblich (1927), the testing of green vegetables on pigeons tends to be misleading since the birds have difficulty in eating enough of the bulky material for adequate nutrition. The following table, reproduced from the *Report of the Medical Research Council*, 1924 (Special Report Series, No. 38), gives a summary of the estimates obtained by Osborne and Mendel (1919, 1920, 1922), Steenbock and Gross (1919), McCollum, Simmonds, and Parsons (1918):

			Percentage required in Diet of Rat.	Daily Dose for Rats (dry weight).	Approximate Equivalent (dry weight) of 1 gram Dried Yeast.
Yeast (dried)	3 to 6 per cent.	0.1 to 0.2 gram	—
Potato	Present, less than clover	>1	—
Sweet potato	Less than carrots	—	—
Carrot	15 per cent. of diet is adequate	>1	—
Swede		>1	—
Dasheen		>1	—
Turnip		>1	—
Onion...		>1	—
Beet leaves and stems	—	4 grms.	—
Beetroot	—	>1	8 grms.
Dried spinach	10 per cent.	>1	—
Dried cabbage	15 per cent.	>1	—
Lettuce	—	0.4 gram	—
Asparagus	—	0.2 gram	—
Celery	—	0.5 to 0.8 gram	—
Parsley	—	>0.4 gram	—
Dandelion leaves	—	>0.6 gram	—
Tea decoction	Absent	—	—
Lucerne (alfalfa)	—	1 gram	—
Clover	—	1 gram	—
Timothy hay	—	>1 gram	—
Sugar beet	No protection when 25 per cent. of diet	—	—
Mangold		—	—

(1) **Spinach.**—According to Sherman (1927), spinach has a vitamin B value of 20 to 24 units per ounce compared with tomatoes 8 to 16 units and potato 8. Sun-dried spinach, compressed and packed in air-tight tins, was found by Shorten and Roy (1919) to afford complete protection from polyneuritis when given in dosage of 5 grms. per day to fowls. Eddy, Kohman, and Carlsson (1925) have reported a lessened potency of dried cooked spinach, as compared with dried raw spinach. The loss is attributed by Yokomaki (1929) to the use of carbonate of soda. They found that canned spinach was as good as the home cooked, and that spinach is a much better source of vitamin B₂ than B₁.

(2) **Celery.**—The employment of ethylene for the artificial ripening of some fruits and vegetables has led to the investigation of the vitamin content of celery so ripened. According to Babb (1928) the results of feeding young rats on known quantities of ethylene and board-blanching celery indicate that the treatment is not injurious to its vitamin B content.

(3) **Turnip.**—A curative action of the juice of raw turnip in 5 to 10 c.c. dosage has been reported by Horvath (1927). Oral administration proved beneficial in mild cases of polyneuritis, but had little or no effect in severe cases, though it was effective in alleviating the severe enteritis of polyneuritic pigeons. There was little evidence of protective action. Horvath suggests that some of its curative action may be due to its effect on oxidations and on blood cholesterol and sugar.

(4) **Potatoes.**—According to Chick and Hume (1916) the potato is not a very good source of vitamin B₁, 350 grms. daily being required to effect the cure of polyneuritis. Sherman (1927) also finds it to contain only 8 units per ounce as compared with spinach, 24 to 30. Sweet potato leaves and shoots, according to Santos and Collado (1928), are a good source of the antineuritic vitamin.

(5) **Some Tropical Vegetables**—(a) **TARO AND POI.**—Taro (*Colocasia antiquorum* var. *esculenta*) is used by the Hawaiians in the form of poi made by cooking the taro and pounding it into a smooth paste with the addition of a small amount of water.

According to Miller (1927), rats fed on sour poi showed a greater consumption of the basal diet than those on fresh poi, probably due to stimulation of appetite by organic acid. Two grms. of taro contained a unit of vitamin A (Sherman's quantitative method), or this amount of taro fed daily caused a gain of about 25 grms. in 8 weeks in a standard rat. A little more than 1 gram of taro contained a unit of vitamin B—an amount which resulted in the same weight of a standard rat at the end of 8 weeks as at the beginning of the experiment.

(b) **YAUTIA AND PLANTAIN.**—The plantain (*Musa paradisaica*) tested on rats by Cook and Quinn (1928) was found to contain 0.3 unit in 1 gram, about the same content as carrots, white potatoes, and turnips. The white and yellow yautia (*Xanthosoma caracu* and *Xanthosoma sagittæ folium*) contained 0.6 and 1.0 unit respectively. (These plants were not tested for the antineuritic factor separately.)

(c) **TANNIA.**—According to Clark (1929) this tuber, which is a common article of diet in Trinidad, is a good source of vitamin B₁ since rats fed on it exclusively develop no signs of polyneuritis. They die, however, from the effects of a water-soluble thermostable toxic principle, with enormous distension of the cæcum, progressive paralysis, and nephritis. The above findings were confirmed by the author (unpublished experiments, 1929).

(d) **SOME PHILIPPINE VEGETABLES.**—The vegetables studied (using white rats) by Santos (1926) were paco (*Diplazium esculentum*, Swartz), balunsay (*Celosia argentea*, L.), and uray babæ (*Amaranthus viridis*, L.). Paco and uray babæ contained only negligible amounts of vitamin B, the alcoholic extract of 2 grms. dry material failing to support growth. Balunsay in the same amount maintained weight for considerable periods and is a better source of vitamin B.

(e) **ALGARROBA (LOCUST).**—According to Rietti (1929) a meal or flour from the fruit of

the white algaroba (*Prosopis alba*—a type of locust tree) used as food in middle and northern France, and a cooked preparation from black algaroba (*Prosopis nigra*) known as "patay," contain a limited amount of vitamin B. *Basella rubra* (Alugbati) and the powdered leaves of *Capsicum frutescens* are stated by Hermano (1930) to be good sources of vitamin B, while *Vigna sinensis* (sitao) contains some.

FRUITS—(6) **Tomato**.—According to Osborne and Mendel (1920) the tomato is a better source of vitamin B than the potato, while Eddy (1921) finds it equal to the orange but better than the apple, and Hartwell (1922) finds it better than either the apple or the orange. Sherman (1926) estimates its vitamin B value as 8 to 16 units per ounce. Tomatoes ripened by the ethylene treatment, advocated by Harvey and co-workers (1928), have been found by House, Nelson, and Haber (1929) to have the same vitamin B content as tomatoes ripened naturally. According to Nelson and Breese (1930), working on tomato juice, the results of their experiments indicate that vine-ripened tomatoes are preferable to those picked green and treated with ethylene gas to develop the colour characteristic of ripe fruit. No indication was observed that the ethylene treatment had any deleterious effect upon vitamins already developed.

(7) **Orange**.—The edible part of orange is stated by Osborne and Mendel (1920) to be as potent as a similar volume of cows' milk. Sherman (1927) also found orange juice to contain 9 units per ounce, as compared with milk 9 units.

In Willimott's experiments (1928) 5 c.c. of orange juice daily were insufficient to supply rats with vitamin B, but when 10 c.c. were given there was little difference between these animals and the controls. The rind also contains small amounts.

(8) **Lemon**.—According to Sherman (1927) lemon juice contains 10 units per ounce of vitamin B. The rind, extracted with 90 per cent. alcohol, has been shown by Willimott (1926) to contain appreciable amounts.

(9) **Grape Fruit**.—According to Willimott (1926) vitamin B is present in considerable amounts in the fresh flavedo.

(10) **Apple**.—According to Osborne and Mendel (1920) the apple is not rich in vitamin B, but contains it. Both Eddy (1921) and Hartwell (1922) found it not so potent as either orange or tomato.

(11) **Banana**.—The banana has been shown by Sugiura and Benedict (1918, 1920) to be a comparatively poor source of vitamin B. According to Eddy (1927) 8 to 10 grms. of ripe banana per day prevents polyneuritis in rats.

(12) **Dried Fruits**.—Currants and dates were found almost devoid of vitamin B by Chick and Hume (1917).

Dried Prunes are estimated by Sherman (1927) to contain 38 units per ounce—a good source when compared with milk, 9 units.

(13) **Grapes**.—Osborne and Mendel (1920) found grapes not so good as oranges, and Randoin (1928) found fresh grape juice, tested on pigeons, to contain very little of the antineuritic vitamin.

(14) **Papaya**.—Ripe papaya (*Carica papaya*, L.), grown in Florida, is stated by Miller (1926) to contain small amounts of vitamin B.

(15) **Peach**.—According to Eddy and co-workers (1926) peaches are very low in vitamin B content.

(I) **MALT**.—Malt and malt extracts are stated by Randoin and Lecoq (1927) to be a good source of vitamin B, though Cooper (1912) found no cure of polyneuritis in doses of 5 to 10 grms.

Vitamin B is stated by Bacharach and Allehorn (1928) to be abundantly present in certain commercial malt extracts, probably being derived from the original unmalted cereal.

With the best extract examined 1.0 gram per day restored growth in young rats on a B-deficient diet.

(J) **BEER AND WINE.**—Both beer and wine seem to be very low in vitamin B content. According to Scheunert and Schieblich (1927) dark beer prepared from rye seedlings and tested on rats and pigeons had a very small vitamin B content, even smaller than that found in porter.

Randoin (1928) states that white wine contains very little vitamin B₁, though a considerable amount of the nutritional vitamin. After partially concentrating the wine to remove alcohol, no vitamin B could be detected, though the syrupy liquid obtained by concentrating the wine to 1/33 of its original volume and filtering was found to be capable of considerably increasing the length of life of rats when added to an otherwise vitamin B-free diet.

(K) **HONEY.**—Early experiments, *e.g.* by Caillas (1926), seemed to show that honey was a good source of vitamin B₁; but later tests, confirmed by Hoyle (1929), show that honey is deficient, and that the deficiency is not due to deterioration consequent on treatment or storage.

(L) **TIKI-TIKI EXTRACT.**—Tiki-tiki extract was recognised in the early days of beri-beri investigation in the Philippine Islands as a valuable curative agent. Recently an attempt has been made by Lara and Nicolas (1930) to establish a definite dosage of this preparation. They estimate its curative dose for pigeons at from 1.3 to 6 c.c. daily, as compared with 27 to 30 c.c. of tomato juice, or 0.75 to 1.5 gram of dry brewers' yeast.

VITAMIN B₂ (Vitamin C₁ of American writers)

While the vitamin B₂ part of the vitamin B complex has been designated, according to the work of Goldberger (1926), "antipellagic," as compared with vitamin B₁ "antineuritic," it must be understood that there are still workers who do not agree that the connection between human pellagra and vitamin B₂ has been definitely established. Irrefutable evidence, however, has shown that the accessory food factor present in comparative abundance in yeast includes a thermostable substance other than the antineuritic vitamin which is destroyed by autoclaving at 120° C. This factor promotes growth and cures, and prevents dermatitis in rats, consequently it has been regarded as identical with the "P.P." factor described by Goldberger and others as curative and preventative of human pellagra.

Although the prevention or cure of polyneuritis in pigeons has been used by many workers in the past as a test of the vitamin B value of any foodstuff, the rat growth method has also been found to give satisfactory results. Foods which have been shown to possess a high vitamin B value by this method must now be understood to be valuable sources of both the antineuritic and antipellagic factors. Since both these factors are essential to growth, a food which is poor in either would give a low vitamin B value when tested by the rat growth method. Such a food has now to be tested separately, as described by Eddy (1927), for its quantitative content of each factor. Similarly, a food which is a good source of both may be much richer in one than the other. Whole wheat, for example, has been shown to be especially rich in antineuritic, and milk in antipellagic value. The general nutritive value of each of these foods has been shown by Sherman and Axtmayer (1927) to be increased, when eaten together, by their supplementary relationship.

LIV. SOURCE OF VITAMIN B₂.

The distribution of the growth-promoting factor appears to be similar to that of the antineuritic. Yeast is a good source of both, and their difference in behaviour towards heat has been used as a method of separating them. In cereals vitamin B₂ is present in less con-

centration than B_1 , and in some of the pulses, such as peas, the B_2 content is low. Fresh milk and meat, egg-yolk and liver, are, on the other hand, much richer in B_2 than B_1 .

LV. CONCENTRATION OF VITAMIN B_2 .

(A) **AUTOCLAVING.**—When yeast is autoclaved at 120° C. at 15 pounds pressure for 15 minutes, the greater part of its antineuritic vitamin is destroyed, while it remains still relatively rich in vitamin B_2 .

Chick and Roscoe (1928) use as the source of vitamin B_2 for their experiments yeast which has been autoclaved for 5 hours at 120° C. This process destroys about half of the original vitamin B_2 present, as well as the vitamin B_1 .

(B) **PRECIPITATION BY LEAD ACETATE.**—An extract of rice polishings precipitated by lead acetate has been found a convenient source of vitamin B_2 by Rosedale (1927). A very concentrated antineuritic extract was first obtained from rice polishings by extraction with 1 per cent. acetic acid and concentration *in vacuo*, until 1 c.c. of concentrate was equivalent to 1 gram of rice polishings; 5 c.c. of this concentrate cured polyneuritis in pigeons in a few hours, and 2 c.c. daily produced steady growth and ensured maintenance. Lead acetate was added to this extract, and the filtrate and residue concentrated so that 1 c.c. = 1 gram of original rice polishings. The filtrate possessed the same antineuritic potency as the original concentrated extract, while the precipitate possessed very little. (It should be noted here that Rosedale (1929) has suggested that the second factor isolated in the precipitate was not the antipellagric factor, but a third factor which prevented symptoms of intestinal stasis. His supposition has not been confirmed.)

Chick and Roscoe (1929) have modified the process, using an extract of washed yeast cells with acetic acid. They found the removal of the antineuritic vitamin more complete in neutral or slightly alkaline solution. Using certain precautions they were able to obtain a clear solution of vitamin B_2 by decomposing the lead acetate precipitate with hydrogen sulphide. The result was not entirely constant, and seemed to depend on the relative amounts of the two vitamins present in the original yeast.

(C) **ADSORPTION ON FULLER'S EARTH.**—It was pointed out by Williams and Seidell (1916) that in fuller's earth, under the same conditions, the growth-promoting factor is more completely adsorbed than the antineuritic.

Using an alcoholic extract of brewers' yeast, Randoin and Lecoq (1928) have attempted to separate the two factors by selective adsorption on fuller's earth. (The "nutritive factor" of these workers is not necessarily the same as the antipellagric factor of Goldberger.) The alcohol was distilled off under reduced pressure and the residue was divided into two parts. One part was dissolved in 15 parts of distilled water, filtered, and the solution stirred with two successive portions of fuller's earth. From the results of their feeding experiments on pigeons they state that the fuller's earth takes out practically the whole of the antineuritic factor from the solution, together with some of the nutritional or growth factor. The aqueous filtrate after evaporation contained only the latter factor. The second part of the residue from the alcoholic extract of yeast was redissolved in alcohol at 70° , filtered, and stirred with its own weight of fuller's earth, added in two successive portions. This fuller's earth fraction was found to contain almost exclusively the antineuritic factor, while the filtrate, after removal of the alcohol, contained only the nutritional or growth factor.

This method has also been employed by Narayanan and Drummond (1930). By fractionation with alcohol they have obtained a concentrate of which the daily dose necessary for the requirements of the young rat contains 6 mgrms. of organic matter.

LVI. PHYSICAL PROPERTIES OF VITAMIN B₂.

(A) **SOLUBILITY**—(1) **In Water**.—Vitamin B₂ is as soluble in water as vitamin B₁.

(2) **In Alcohol**.—According to Chick and Roscoe (1929) and also Sherman and Sandels (1929), vitamin B₂ is insoluble in alcohol of 92 per cent. by weight, which dissolves vitamin B₁. The fact that alcohol treatment appears to destroy vitamin B₂ prevents it from being separated from B₁ by the use of alcohol.

(3) **In Acetone and Benzene**.—According to Pryde (1928) B₂ is less soluble than B₁ in acetone and benzene.

(B) **ADSORPTION**.—On animal charcoal and fuller's earth vitamin B₂ is more completely adsorbed and more difficult to recover after adsorption than B₁ (Seidell (1916), Randoin and Lecoq (1928), Narayanan and Drummond (1930)).

According to Salmon, Guerrant, and Hays (1928) the optimal adsorption of vitamin B₂ by fuller's earth occurs at pH 0.08, the most acid solution tested. It decreases gradually to pH 6.3, and thence remains approximately constant to pH 12.07, the most alkaline solution tested.

(C) **DIALYSIS**.—Chick and Roscoe (1929) state that vitamin B₂ dialyses through cellophane as freely as does vitamin B₁.

(D) **STABILITY**—(1) **To Heat**.—Vitamin B₂ has been called "thermostable" because it remains undestroyed by autoclaving at 120° for 4 or 5 hours—a process which destroys vitamin B₁. It is pointed out by Eddy (1928) that vitamin B₂ is only relatively thermostable. If the autoclaving is continued for a longer time the antipellagric power of yeast also disappears. Vitamin B₂ is therefore capable of destruction by heat, but far more resistant to it than vitamin B₁.

(2) **Acids**.—According to Narayanan and Drummond (1930) vitamin B₂ is stable to acid.

NITROUS ACID.—It has been stated by Levene (1928) that, unlike vitamin B₁, vitamin B₂ can be destroyed by treatment with nitrous acid.

Chick (1929) has failed to confirm this observation. A vitamin B₂ preparation from yeast was found to possess equal power, before and after nitrous acid treatment, to induce growth in young rats on a diet otherwise free from this vitamin. Narayanan and Drummond (1930) also find vitamin B₂ stable to nitrous acid.

(3) **Alkalies**.—According to most workers, including Hassan and Drummond (1928), Narayanan and Drummond (1930), vitamin B₂ resists treatment with alkali, which destroys vitamin B₁. Recent work by Chick and Roscoe (1930), and Chick and Copping (1930), however, appears to show that vitamin B₂ is sensitive to alkali at high temperatures. In acid solution with the pH maintained at 5, no loss in potency could be detected after incubating for 2 hours at 90° to 100° C.; but 50 per cent. loss occurred after incubating an acid solution (maintained at a pH of 5.0 to 3.0) from 4 to 5 hours at a temperature of 123° C. When the reaction was alkaline—namely, with a pH of 8 to 10—the loss of potency was 30 per cent. after 10 days at room temperature, 50 per cent. after 2 hours at 90° to 100° C., and 75 to 100 per cent. after 4 hours at 123° C. These workers suggest that the statement of other observers, particularly Reader (1930), that yeast contains a third factor, thermo-alkalilabile, is unjustified.

(4) **Radium**.—Sugiura and Benedict's observations, made as early as 1919, suggested that inactivation of the growth-promoting factor in yeast occurred as a result of exposure to radium emanation.

LVII. PHYSIOLOGICAL ASPECTS OF VITAMIN B₂ DEFICIENCY.

In applying the term "antipellagric" to the B₂ factor of the vitamin B complex it must be remembered that the application cannot be recognised in a strictly limited sense. While

this factor tends to the development in animals of symptoms which resemble pellagra, many workers refuse to admit that vitamin B₂ is strictly antipellagric in human beings in the sense that vitamin C, for instance, is antiscorbutic.

Dietary means for the prevention of pellagra are, however, being used in regions where pellagra used to be prevalent. The increase in the incidence of pellagra in the spring of 1927 in the Southern States was attributed by Goldberger and Sydenstricker (1927) to the destruction by the Mississippi floods of the sources of dairy produce and fresh vegetables. Similarly the mill village region of Western South Carolina is quoted in the *American Journal of Public Health* (May 1929) as a place where "balanced diet" is regarded as the sovereign remedy and preventive.

(A) **PELLAGRA**—(1) **Etiology**.—The first authentic mention of pellagra as a specific disease was in 1762 when, under the title of "Mal de la Rosa," Don Gaspar Casal, physician to Philip V. of Spain, described it as having occurred in Spain in 1735. In 1740 the disease was recognised in Italy, but Sambon (1912) states that the first person to describe it under the popular name of "pellagra" (rough skin) was Francesco Frapolli in 1771. From that time onwards the disease was constantly described in Italian literature. In 1890 the first "pellagrosario" was erected at Inzago, near Milan. Lavinder (1912) stated that the disease reached its zenith in Italy in 1881 when the total number of cases was 104,067; by 1910 this number had decreased to 33,869.

It was not until 1907 that pellagra became a recognised subject of inquiry in the United States, though it must have existed there for many years previously. Lavinder stated in 1912 that over 30,000 cases were estimated to have occurred since 1908, while Babcock (1912) considered the disease to be a national problem, accounting for 30,000 cases of illness in the United States at that time.

In the British Isles the first cases reported were by Billod in 1865; these cases—two brothers—had been brought to his notice in 1860 by the Inspector-General of the Scottish Lunatic Asylums. The incidence of a few isolated cases in the British Isles from this time onwards, together with the investigation of Sambon and Chalmers (1912) in Scotland, is described in detail by Bigland (1923). In 1916, Niles reported 1845 cases in Mississippi in the last 3 months of 1914, and in 1918, according to Osler, there were probably 100,000 cases in Italy and 50,000 in Roumania. In 1920 an outbreak among the German prisoners of war in Egypt, with a total number of 65 cases, was described by Enright. An idea of its prevalence in America can be obtained from Garrison's estimate (1928) of 5000 to 10,000 cases, with approximately 500 deaths per annum in a population of 1,800,000 in Arkansas, and from Goldberger's statement (1929) that "on a conservative estimate 120,000 people in the United States suffered from an attack of pellagra in 1927."

THEORIES OF ETIOLOGY OF PELLAGRA

(a) **THE MAIZE THEORY**.—The Italian school supposed that pellagra was due to poisoning by maize (*Zea mais*). Some of the investigators in this subject, especially Lussana, Strambio, and Albertoni (1914), believed the bad influence in maize to lie in its insufficient nutritive value.

The "toxic zeistic" theory, originated by Sette and Balaidi, and later associated with the name of Lombroso (1871), stated that pellagra was probably due to toxic substances found in sound or in spoilt maize; (2) special toxins produced by certain moulds which develop in maize under particular conditions; (3) the increase in virulence of *Bacillus coli* in the intestine attributable to a maize diet, although the corn is in good condition; (4) the action of definite pathogenic agents introduced into the organism at the time of the ingestion of the maize. Upon these principles and theories were based all the Italian legislative measures for the prevention and cure of pellagra.

A theory originated by Raubitschek and others (1912) supposed pellagra to be a photo-dynamic disease: the fatty and colouring matters of maize were believed to sensitise the tissues to the action of light, and so to give rise to the skin eruptions. This theory was not confirmed by Rondoni (1911), nor would it explain the common occurrence of new cases in February and March, when the sun's rays are weak. Other researches were directed towards the study of general and local reactions of pellagra patients to the injection of maize extracts under the skin.

Volpino (1912) and others reported a violent reaction in pellagrous subjects, while Rondoni (1912) found a less definite enhanced sensibility which he thought might be interpreted as an anaphylactic reaction to some unknown and therefore undefined factors of the maize extract.

In 1915 Cencelli summarised the facts which led to the abandonment of the maize theory as follows:

"There are many sufferers from pellagra who have *never* lived on maize; entire districts in Italy (Molise, Campania, Basilicata, Calabria, Liguria) and abroad (Brazil) where good, mediocre, and bad maize are used to an extraordinary degree, in fact almost exclusively, are free from the disease, while in some provinces and communes where pellagra is common, immune zones exist, the latter being clearly marked off from the infected areas, although the climatic, economic, hygienic, and dietetic conditions are identical, and maize is the staple article of food. In a family where the members must more or less have inherited the same tendencies, and are subject to similar hygienic and dietetic conditions, and to the effects of equally excessive toil, one or two (rarely more) sicken.

"The prophylactic and curative measures hitherto adopted, and which were based upon the maize theory, have in truth yielded but little result. The amount of foreign maize (which is considered the most to be feared) imported into Italy has no effect upon the increase or decrease of pellagra. In spite of the *absolute* abolition of this cereal as an article of diet the disease may continue to show itself with progressive symptoms for many consecutive years."

These facts were confirmed in 1922 by the results of the issue of 7,000,000 bushels of maize to Russia by the American Relief Administration. Mainly because the money available for relief in Russia would buy two and a half times as much maize as any other cereal, it was decided to risk the chance of pellagra, for, as one of the administrators expressed it, "corn (maize) was the sole hope against death; Russia had got beyond the fear of disease." In the result no outbreak of pellagra occurred; in the whole of the Volga famine district not a single case was reported. In Ukraine there were 20 suspected cases which were specially investigated. Of these only 4 were found to be suffering from true pellagra, and not one of the 4 had eaten any of the American corn. The supplementary food of the Russian peasants was negligible, for previous to the arrival of the corn they had existed on the bark of trees, clay, weeds, and even human flesh. With maize as their sole article of diet, nevertheless, they did not develop pellagra.

The recent investigations of Mellanby (1930) suggest, however, that the connection between maize and pellagra may not be entirely lacking. Comparing the nerve degenerations in the cord of pellagrins with those of nervous ergotism, which he says are produced by a toxic factor antagonistic to vitamin A preventable by foods rich in vitamin A, Mellanby suggests that pellagra may be associated with a "toxamin" present in maize and antagonised by foods containing a protective agent of the nature of vitamins B₂ or A, or both.

(b) THE INFECTIVE THEORY—(i) *The Simulium Fly* (Sambon, 1913).—At the Tropical Section of the British Medical Association at the meeting in Leicester in 1905, Sambon first pointed out that pellagra did not seem to be a food disease or due in any way to unsound maize, and suggested that in all probability it was protozoal in origin.

In 1913 an English Commission, with Sambon at its head, left England to investigate the pellagra problem in the West Indies. Sambon's researches led him to conclude that maize

had nothing to do with pellagra; that pellagra had a definite topographical distribution; that its endemic foci might remain unaltered for at least a century, and were closely connected with running water; and that a blood-sucking insect (*Simulium*) probably conveyed it. At one time he thought the parasite might be a trypanosome, later an ultramicroscopic virus. Later still (1914), instead of incriminating only the *Simulium*, he included the Chironomidae and Ceratopogoninae—flies whose natural history is still but little known.

(ii) *The Thompson McFadden Report*, 1913.—The leading investigators in the Thompson-McFadden Pellagra Commission, which studied the food conditions and hygienic environment in the cotton mill districts of South Carolina, were Siler and MacNeal (1914, 1916, 1918). They concluded that pellagra was in all probability a specific infectious disease, communicable from person to person by means at present unknown, but suggested a blood-sucking insect (*Stomoxys calcitrans*) as a probable carrier. They stated that they had discovered no evidence incriminating flies of the genus *Simulium*, except their universal distribution throughout the area studied.

In later reports (1916 and 1918) they still held the theory that improvement in sewage disposal tended to prevent the spread of pellagra.

(iii) *Fungi (Aspergillus, etc.)* (Ceni, Jobling, etc.)—Ceni (1913) believed that pellagra was an infection with *Aspergillus fumigatus* or *A. flavescens*, or, rarely, with both; later he added *Penicillium glaucum* to the list as especially active in the chronic cases of pellagra.

Jobling and co-workers (1917, 1923) have also presented evidence that a fungus of the *Aspergillus glaucus-repens* group is the infectious agent involved. Their theory includes, however, the proviso that this agent can only establish itself in the human organism when malnourished by an unbalanced diet.

(iv) *A Streptobacillus* (Tizzoni).—Tizzoni (1913, 1914, 1915, 1916) attributed pellagra to the pleomorphic *Streptobacillus pellagræ*, which he described in detail. He stated that he had isolated it from the circulating blood and the faeces of pellagrous patients, and also in spoilt maize. According to him it had several forms, including an intermediate or actinomycotic, and a still higher or hyphomycetous form.

Perroncito (1913), however, failed to find it in the blood of 20 severe cases of pellagra, and other investigators, including Bigland (1920), were equally unsuccessful.

As recently as 1926, 1927, and 1930 Susman has claimed to have isolated from the blood

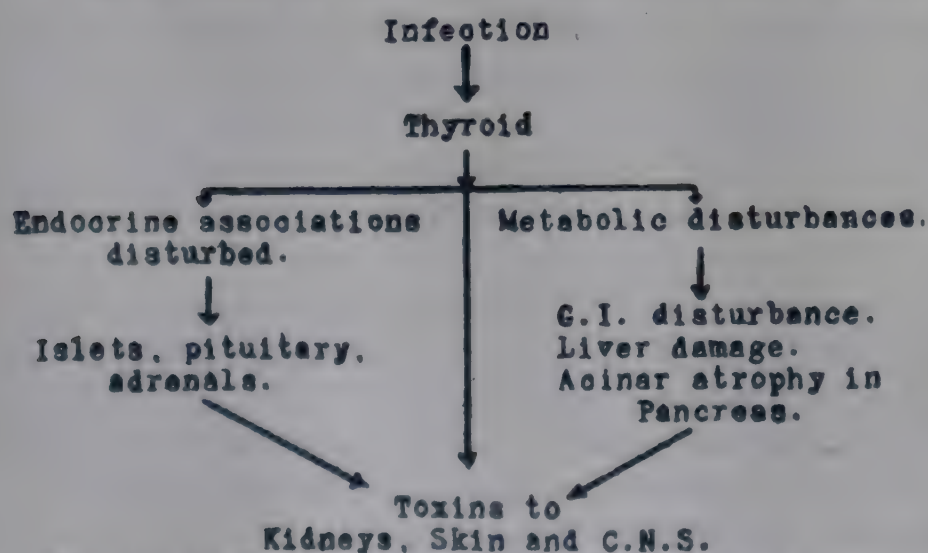


Diagram to illustrate the suggested Process in Pellagra.
Susman (1930).

of 4 patients suffering from pellagra an organism which grows anaerobically in glucose broth, and when treated with Loeffler's methylene-blue presents the appearance of a bacillus with deeply staining polar structures.

While not definitely claiming that this organism is responsible for the production of pellagra (he states, in fact (1930), that the lesions do not suggest the presence of a virulent organism), Susman suggests that a blood-borne infection may be the cause of the disease.

The process suggested is a primary disturbance of the thyroid, in which both an organism and a dietetic factor may be concerned. This thyroid lesion may give rise to a disturbed digestive mechanism and to an endocrine maladjustment. The endogenous toxins so liberated may produce lesions in the kidneys, skin, and central nervous system.

(v) *A Coccus*.—Salmon, Hays, and Guerrant (1928) claim to have found a Gram-positive coccus constantly associated with pellagra. They state that this organism has been isolated (often in pure culture) from skin lesions, arthritic lesions, parenchymatous organs and walls of the intestines of pellagrous rats. With two exceptions the organism has not been obtained from the blood stream. The organism has been fed to rats, and the characteristic lesions, from which the organism has been recovered, have been produced. They find, however, that there is a relation between the character of the diet and the occurrence of the syndrome, and have prepared concentrates of a protective principle (P.P. factor) from *Pueraria Thunbergiana* (*leguminosæ*) which cure or prevent the occurrence of the disease. A relatively low concentration of these preparations in nutrient broth inhibits the growth of the causative organism.

(c) THE CHEMICAL THEORY OF ALESSANDRINI (1912).—Alessandrini and Scala brought forward the theory that pellagra is contracted in definite zones where the usual drinking water comes from springs arising in argillaceous soil or from the streams and stagnant pools in a clay district. This theory was provisionally accepted by the Pellagra Commission of the Province of Rome in preference to any theories connected with maize or the biting of insects. It was urged by Alessandrini and Scala that pellagra was a disease induced by chronic poisoning brought about by the silica in colloidal solution in waters of determined composition; in other words, that pellagra was attributable to mineral colloids. They supposed further that human pellagra was an acidosis and could be cured or greatly improved by administering neutral sodium citrate.

According to Cencelli (1915) the Pellagra Commission of Rome was so impressed by these observations that they attempted the correction of the water by means of the introduction of pieces of lime in the commune of Onano (Viterbo), which is the part of Lazio where pellagra has always been persistently endemic. The aqueduct was completely restored, and all possible sources of contamination were removed, while a number of minute pieces of calcium (previously well washed) were introduced into the reservoir and along the channel.

(d) THE DIETARY DEFICIENCY THEORY.—The earliest descriptions of pellagra associate it with insufficient diet. Pedro Casal wrote in 1707 describing pellagra among peasants: "Their food is maize, milk, chestnuts, herbs, and nuts, and the patients have obtained great relief when their food has been replaced by other substances of a more sustaining kind."

With the more or less wide acceptance of the infective theory the deficiency aspect of the problem received little attention until Goldberger began his extensive investigation in 1914. From that time onwards the question of food deficiency as a cause of pellagra resolved itself into two separate aspects—a deficiency of protein, or a vitamin deficiency.

(i) *Protein Deficiency*.—In his earlier observations Goldberger was inclined to attribute the dietary defect to a deficiency of protein not only in quantity but in quality. He first drew attention to the well-known fact that attendants upon pellagrins do not contract the disease, although many chronic lunatics develop it after confinement in an asylum for 10 or more years. He considered that the exemption enjoyed by nurses, attendants, and employees was due to their having a more varied diet, even when their rations were supposed to be the same as those of the lunatics. He urged that the dietary of those among whom pellagra is most prevalent should be improved, and that in the Southern States, fresh meat, eggs, and milk should be substituted for "cereals, vegetables, and canned foods, that enter to so large an extent into the dietary."

Later (1915) he placed 11 convicts on a dietary which was lacking in fresh animal protein, with the result that within 5 months six of the 11 developed pellagra. The converse experiment was then made in an orphanage where pellagra made its appearance every year. He found that 75 per cent. of the children were pellagrous in 1913, and the ones who escaped were the younger children whose diets were supplemented with cows'milk. His suggestions of a

diet reform were adopted, and, in 1914, in the same institution among 234 inmates there did not occur a single case.

On the basis of these observations Goldberger, Waring, and Willets (1914) decided that pellagra was neither infectious nor contagious, but essentially of dietary origin, and "that it is dependent on some yet undetermined fault in a diet in which the animal or leguminous protein component is disproportionately small and the non-leguminous vegetable component disproportionately large."

In an attempt to disprove the infective theory, Goldberger then (1916) examined the evidence for the transmissibility of pellagra, and also tested its supposed infectivity himself by experiments on human beings.

In 1913 Harris's claim to have successfully inoculated a monkey with a filtrate from pellagrous lesions, seemed to furnish direct evidence that the disease was communicable. Later observations, however, including those of Harris himself, also of Lavinder, Francis, Grimm, and Lorenz (1914), failed to confirm his results.

Dearman (1914) also claimed that, after injecting rabbits with pellagrous blood, in 9 days symptoms developed, and in 23 days erythema, desquamation, and pigmentation of the skin followed.

Bigland (1920), however, failed to find any ill-effects from intravenous injections into a rabbit of 2 c.c. of pellagrous serum at 2-day intervals.

In Goldberger's attempts (1916) to transmit pellagra to human beings he used material obtained from 17 pellagrins and consisting of blood, naso-pharyngeal secretions, epidermal scales from pellagrous skin lesions, urine, and fæces.

Scales, urine, and fæces were administered by mouth; the blood was administered by intramuscular or subcutaneous injection, and the secretions by application to the mucosa of the nose and nasal pharynx. Certain variations were also indulged in. In order to reduce gastric acidity and thus minimise any possible germicidal action of the gastric juice, the ingestion of material was preceded by a dose of sodium bicarbonate. The products were always taken on an otherwise empty stomach. The fæces, urine, and scales were often administered together in the form of a pill made up with flour or bread crumbs.

The results strongly supported the view that pellagra was not infective for, in spite of the procedures indicated, no symptoms of pellagra resulted in any case.

Goldberger (1916) therefore claimed to have proved definitely that pellagra was the result of a deficient or ill-assorted diet, and that the only treatment for the disease was dietetic. He stated that the available data were not sufficient to furnish any definite evidence as to the exact nature of the mechanism involved in causing or curing the disease, but emphasised the fact that "pellagra results from a fault in the dietary and can be prevented or cured by including in the diet suitable amounts of leguminous protein substances and fresh animal food." From this standpoint he continued to view pellagra for the next few years.

In 1922 he, with Tanner, had come to the conclusion that the character of the protein was the important factor. Since cases of pellagra had been reported to have occurred when the diet was judged to have contained a liberal supply of mineral elements and the known vitamins, they assumed that these factors could be excluded. They concluded, therefore, that "the essential etiological dietary factor is a specific defect in the amino-acid supply, probably in the nature of a deficiency of some special combination or combinations of amino-acids." In some preliminary therapeutic trials with amino-acids they found that the dermal lesions in each of 2 cases seemed to show a markedly favourable reaction to cystine; and in a third case a steady gain in weight, with some improvement in diarrhoea, accompanied the administration of both cystine and tryptophane.

In 1924 Goldberger and Tanner investigated the effect of various foods on the course of

pellagra and found that fresh meat and milk were the most satisfactory remedies. They estimated that about 4 to 4½ ounces of fresh lean beef and 40 ounces of buttermilk would suffice to prevent pellagra in all but very exceptional instances. Fresh butter and cod-liver oil were found to be unsatisfactory as preventive agents. While still holding to the "faulty protein" theory they now added to their conclusions the possibility of "a deficiency in some as yet unrecognised dietary complex, possibly a vitamin."

In the meantime other workers had brought forward evidence of the connection between dietary deficiency and pellagra. Sandwith (1915) found that early cases of pellagra in Egypt could always be cured by admission to hospital and by an abundant mixed diet.

Wilson (1919) directed attention to the "biological value of protein" in cases of pellagra. He pointed out that meat protein is much more readily assimilable than vegetable protein, and that it is the assimilability of protein which constitutes its biological value.

Wilson employed Thomas's tables (1909) for determining the values of the different proteins. Thomas defined the biological value of protein as the number of parts of body nitrogen replaceable by 100 parts of the nitrogen of the foodstuff. His method consisted in remaining for a few days on a nitrogen-free diet until his nitrogen output fell to a low figure, taken to represent only endogenous metabolism, and then testing the power of various proteins, fed as additions to the nitrogen-free diet, to replace the nitrogen lost in tissue destruction. He estimated that the amount of tissue repair from 30 grms. of meat or milk protein required 100 grms. of maize protein to be equalised, and the upholders of the protein deficiency theory believed that the inferiority of maize proteins could be explained by the large proportion of zein, which is devoid of both tryptophane and lysine—two amino-acids regarded as essential for animal nutrition.

Wilson reported that "pellagra occurs or does not occur according as that value was less or more than 40 grms. per diem." The pre-capture diet of the Turks on the Sinai front showed 30·4 as the biological value of the protein; in 1918 on the Palestine front it fell to 21. Some were pellagrous when captured, and some developed pellagra after capture.

Boyd and co-workers (1919–1920) studied pellagra in Egypt among prisoners of war. They found that the level of biological protein could be so exactly determined that they were able to anticipate the disease.

They also pointed out that a diet which was adequate to prevent pellagra while the individual was resting was inadequate when he was put to work. Among 2000 German prisoners, who were able to supplement the prison diet in various ways, none developed pellagra. Among 6000 Ottomans who were restricted to the prison diet, 300 cases developed in one year. It was found that if the disease was not far advanced it promptly responded to an increase in the biological protein value. These workers emphasised the importance of the part played by diarrhoeal disease in disturbing the protein balance.

In 1920 Enright advanced opposition to the food deficiency theory on the ground that during the pellagra outbreak among German prisoners of war in Egypt the prisoners who suffered from pellagra had received a diet ample both in quantity and quality.

Goldberger (1920) replied to his argument with the suggestion that the dietary faults responsible for the outbreak were probably the result either of improper distribution or of individual eccentricities of taste, or both. He advanced the same explanation for Viswalingam's conclusion (1920) that "faulty diet in itself cannot cause pellagra, and that there is a superadded infection." Both this theory and Bigland's toxin hypothesis (1920) Goldberger considered "invoked without substantial grounds" and to be regarded as superfluous. Although 1925 saw a change in Goldberger's views of the causation of pellagra and a tendency to ascribe it to a vitamin deficiency rather than a protein deficiency, other observers, especially Wilson (1921, 1930), still continue to hold the latter view.

Taking the statistics of an outbreak of pellagra in the Durban prison command obtained by Cluver (1929), Wilson (1930) questioned Cluver's opinion that the dietaries which had produced pellagra had done so by virtue of their low vitamin B₂ content. Wilson pointed out that these dietaries might be expected to be pellagrous from the value of the protein content. He considers that the essential cause of pellagra is a primary or secondary deficiency in the value of the protein intake relative to the individual requirement—a requirement which may be raised by various conditions.

(ii) *Vitamin Deficiency*.—Although to Goldberger must be given the credit for the association of pellagra with a specific vitamin deficiency, other workers had already pointed out the similarity between pellagra and diseases, such as beri-beri, known to be due to lack of vitamins. As early as 1914 Funk pointed out that in districts where pellagra was common the food was deficient in the necessary "vitamine." He believed that in localities where maize formed the staple article of diet the deficiency was directly due to the removal of the exterior of the grain by milling, leaving the remainder deficient in "vitamine." He advocated whole rice, potatoes, fresh fruit, and milk as a preventive and cure. This theory was supported by Wood (1916), Vedder (1916), and Weill and Mouriquand (1917).

Wood had followed up Nightingale's work (1912) on the effect of milling corn by steam or electric power. Nightingale described the disease from which prisoners suffered when fed on the milled meal as "zeism," and stated that the substitution of hand-milled corn completely removed the trouble. In 1920 Wood again emphasised, though he did not exclude the possibility of an infectious element playing a part, the striking coincidence in the appearance of the disease with the adoption of modern errors in milling and cooking.

In 1925 there appeared in the *United States Health Reports* an article by Goldberger and Tanner, entitled "A Study of the Pellagra-Preventive Action of Dried Beans, Casein, Dried Milk, and Brewers' Yeast, with a consideration of the Essential Preventive Factors Involved." The results of these observations led them to modify the protein deficiency theory which they had hitherto held to be subject to explain the connection between pellagra and faulty diet, and their conclusions were as follow :

"(a) A liberal supply of protein presumably of good biological quality does not completely prevent, though it may modify, the clinical picture of pellagra by notably delaying or preventing the development of the distinctive dermatitis. This modifying action may be of an indirect, sparing nature.

"(b) In the prevention (and presumably causation) of pellagra there is concerned a heretofore unrecognised or unappreciated dietary factor which we designate as factor P.P. This may be effective with but little, possibly without any, co-operation from the protein factor.

"(c) Factor P.P. may possibly play the sole essential rôle in the prevention (and causation) of pellagra.

"(d) Factor P.P. is present in brewers' yeast, in milk, and (on the basis of our experience with fresh meat) in lean beef ; it is very low or lacking in dry soy beans, dry cowpeas, butter, cod-liver oil, and canned tomatoes."

Continuing their investigations into the pellagra-preventing properties of various food-stuffs they discovered (1926) that dried yeast extract taken in quantities of $\frac{1}{2}$ ounce daily could invariably prevent pellagra, but that even this amount did not always prevent the appearance of beri-beri. The yeast extract used in these experiments was a dried acidulated watery extract containing a very little protein nitrogen. Goldberger and co-workers observed also at this time that the disease of "black-tongue" in dogs, which owes its name to areas of superficial necrosis on the tongue, was capable of being produced and cured by the same dietetic measures as pellagra in human beings. "Black-tongue" appears to have a geographical distribution in the United States similar to that of pellagra, and, like pellagra, it involves a

disturbance of the alimentary tract—stomatitis and diarrhoea. This condition was first observed by Chittenden and Underhill (1917), resulting from the feeding of dogs on a diet deficient in nitrogen. The syndrome described later by Underhill and Mendel (1928) was stated by them not to be cured by yeast, but to be curable by butter and by crystallised carotin.

It is suggested by Aykroyd (1930) that the discrepancy in the results of these two groups of workers may lie in the fact that since Underhill and Mendel's diets were deficient in vitamin A, their syndrome may have had its origin in vitamin A deficiency.

Mellanby's investigations (1930) into the toxic factors in cereals open up a further interesting speculation, which may reconcile the conflicting results. He suggests that maize may contain a toxic factor which may be prevented from exercising its pellagra-producing capacity by the presence of a protective factor of the nature of a vitamin or possibly two vitamins—B₂ and A. Since carotin acts in the same way as vitamin A, though the two have not been proved identical, Underhill's results with butter and carotin may have been due to the neutralising action of vitamin A upon the toxic factor, while Goldberger's with yeast may have been due to a similar action with vitamin B₂.

Goldberger's original experiments have been repeated and confirmed by Chick and Roscoe (1928), Findlay (1928), and others, and the experimental production of a condition in rats characterised by a symmetrical dermatitis and by lack of growth has been placed beyond doubt. The factor determining this condition has been called vitamin B₂ and is supposed to be identical with the P.P. factor of Goldberger.

In 1929 Aykroyd and Roscoe showed that the distribution of vitamin B₂ (rat factor) was roughly similar to that of Goldberger's P.P. factor and to the black-tongue preventive factor.

The following table is taken from Aykroyd's article in the *British Medical Journal* :

COMPARISON OF THE EFFECTS OF VARIOUS FOODSTUFFS ON VITAMIN B₂ DEFICIENCY IN RATS, ON PELLAGRA IN MAN, AND ON BLACK-TONGUE IN DOGS.

Material.	Rat Vitamin B ₂ .	Man P.P. Factor.	Dog Black-Tongue Preventive Factor.
Maize endosperm ...	Very poor	Associated with pellagra in U.S.A. and Italy	—
Wheat flour ...	Very poor	Associated with pellagra in U.S.A.	Used in basal diet to produce the disease.
Whole maize ...	Poor; 50% less than normal growth	Traditionally associated with pellagra	No preventive action, used in basal diet to produce the disease.
Whole wheat ...	Poor, but better than maize	—	66% in diet delayed onset of the disease; no protection.
Peas ...	30-40% needed for good growth ("Clipper" peas)	30-35% (168 grams daily); incomplete protection (Virginian cowpea)	58%; slight protection, 73%; almost complete protection (Virginian cowpea).
Wheat germ ...	15-30% good growth	30%; protection almost complete	31%; protection almost complete.
Fresh milk ...	5-10% milk solids, good growth	20% solids (1200 grms. buttermilk daily) protected	250-300 c.c. skim milk daily protected.
Lean meat ...	7-10% (dry wt.), good growth	10% (dry wt. 50 grms. daily) protected	11% (dry wt.) protected.
Dried yeast ...	1-3% good growth	7% (30 grms. daily) protected	5% protected.
Liver ...	1-2% (dry wt.) ox liver, good growth	Alcoholic extract of ox liver effective in treatment	11% dried pork liver protected.
Dried egg-yolk ...	7-15% good growth	—	17% protected.

In the series of experiments from which the results in the table are drawn, over 60 per cent. of control rats on a basal diet, free from vitamin B₂, but otherwise complete, developed pellagra-like symptoms.

Aykroyd points out, however, that rats do not develop these symptoms on diets similar to those which are associated with pellagra in man and experimental black-tongue in dogs, and are indeed able to grow and remain free from obvious symptoms on such diets. "Only a very highly purified food mixture will induce rat 'pellagra,' and at present the appearance of symptoms is somewhat intermittent. Experiments on rats cannot, therefore, prove pellagra to be a deficiency disease, but the fact that the rat's needs for vitamin B₂, set, perhaps, on a low scale, are satisfied by various foodstuffs in their order of potency as regards the P.P. (pellagra-preventive) factor and the black-tongue preventing factor, is suggestive of the identity of the three factors."

Wilson, using as a basis the relatively high vitamin B₂ value of a sample of whole maize used in Aykroyd's experiments, has argued that all diets containing much whole maize must have a fairly high vitamin value. He states also that comparing non-pellagrous with pellagra-producing foods an assessment on the basis of Aykroyd's results shows that the vitamin B₂ content of the food has no relation to the incidence of pellagra. Aykroyd points out, however, that Thomas's biological values of protein have never been entirely confirmed, and that the distribution of the rat factor is not completely known.

So far, vitamin B₂ has been found most abundantly in foods containing animal protein—that is, eggs, milk, meat, liver, etc.—of high "biological value." Human dietaries are therefore liable to coincide roughly as regards their vitamin B₂ value and the biological value of their proteins. Many diets associated with pellagra, as Wilson says, are low in both respects.

The vitamin (as against the protein) deficiency theory of pellagra rests on the proved possibility of preventing and curing the disease with non-protein substances. The failure of casein to give complete protection against the disease in man and the production in the rat of pellagra-like symptoms on a diet containing this "good" protein, point in the same direction.

All these workers, though their views are divergent in general, agree on one point, that there are many facts regarding pellagra for which a fully satisfactory explanation has not been found.

It appears probable that the relationship between protein requirement and vitamin B₂ content of the diet, as recently investigated by Reader and Drummond (1926) and Hartwell (1928), may have a very close bearing upon the question. These studies have shown that, at any rate in the growing animal, vitamin B₂ is essential to the metabolism of protein in the body, and that the protein requirement is least when it is of the animal variety. Wilson's suggestion "that among individuals living on the border-line of vitamin sufficiency the value of the protein intake would determine the pellagrous or non-pellagrous effect of the diet" seems a feasible one, and the origin of the pellagra-like condition described in rats might well lie in the abnormal metabolism of protein in the absence of the B₂ factor.

Recent investigations by Kollath (1929) have led him to express the view that the identity of human and rat pellagra is not by any means proved.

In studying the factors X (heat-stable) and V (heat-labile) affecting the growth of the influenza bacillus he found points of similarity between X and vitamin B₂, and V and vitamin B₁, and suspected that alkaline hæmatin might act in the capacity of vitamin B₂ as a pellagra preventive. He states that typical beri-beri only appears when the pellagra-preventive factor is present in the diet, and found that this occurred in the presence of alkaline hæmatin and in the absence of vitamin B₁.

It is not known whether the denaturation of the hæmatin has given it the properties of

a vitamin, or whether it is a contaminating substance which has this action, and the problem is being further studied. The effect of alkaline hæmatin in human pellagra remains to be seen.

VITAMIN B₂ AND PROTEIN METABOLISM

In 1924 Hartwell pointed out that there was a quantitative relation between the amount of protein and the amount of vitamin B in the diet which favour normal nutrition.

Hassan and Drummond (1927) confirmed this fact. They found that when the amount of protein in the diet was as high as 70 per cent., yeast extract to the amount of 16 per cent. was necessary to obtain normal growth; whereas if the protein were only about 20 per cent. of the diet, 4 per cent. of yeast extract was sufficient.

Later they found that normal growth followed the addition of yeast extract even when such extract had been treated with hot alkali, thereby inactivating the antineuritic vitamin. They concluded, therefore, that the factor in yeast which resisted the effect of hot alkali was the factor chiefly concerned in rendering high protein diets adequate for growth.

In her later experiments (1928) Hartwell pointed out that different proteins require different amounts of vitamin B in order that they may be properly metabolised. Thus, edestin required more yeast extract than either caseinogen or egg albumen for normal metabolism in young rats.

Another link between vitamin B₂ and the metabolic processes of the body is suggested by the experiments of Kon (1929). He found that rats on a diet deficient in the heat-stable component of the vitamin B complex showed an increased c/n ratio in the urine as compared with individual controls receiving equivalent amounts of a complete diet. This difference was less marked for animals deprived of the whole vitamin B complex and was negligible for those receiving autoclaved yeast.

(2) **Clinical Aspects of Pellagra.**—Osler (1930) defines pellagra as a "deficiency disease with periodical manifestations characterised by gastro-intestinal disturbances, skin lesions, and a tendency to changes in the nervous system."

The seasonal incidence of the disease is one of its most peculiar characteristics. It begins nearly always in the spring, may improve during the summer, relapse during the autumn, recover again during the winter, and reappear at a corresponding time for the next year. This periodicity may show itself for as many as 25 years in succession, the patient being in a worse condition at the end of each attack. The chronic form of the disease has been described by Shelley (1930) as occurring in the following types:

- (a) Subthyroid—with a rough, dry, scaly skin, diffuse pseudo-œdema, and mental apathy.
- (b) Gastro-intestinal—with repeated attacks of dyspepsia, accompanied by diarrhoea or constipation.
- (c) Paralytic.
- (d) Mental—accompanied by delusional or maniac-depressive insanity and occasionally acute mania.

There is an acute form which runs a rapid course, marked by delirium fever and diarrhoea, and which may end fatally in a few weeks.

(a) **THE SYMPTOMS** may affect—(i) *The Alimentary Tract.*—The onset is generally characterised by general malaise with nausea, dyspepsia, vomiting, and very commonly diarrhoea, intractable and dysenteric in type. Aphthous stomatitis and salivation also occur frequently.

(ii) *The Skin.*—The rash is symmetrical, occurring on exposed parts of the body, especially the hands and feet, and accompanied by burning. Following the erythema the skin becomes dry, scaly, and pigmented. Desquamation leaves cracks and fissures and a new red surface. When vesicles form, containing serum, pus, or blood, the name "wet pellagra" is sometimes applied, and the prognosis is bad.

(iii) *The Nervous System*.—Various manifestations of spinal lesions may be present, pointing to a condition of subacute combined degeneration of the spinal cord. Mental symptoms, including depression amounting to melancholia, confusion, emotional and maniacal states, often terminate in a chronic dementia.

(b) PATHOLOGICAL CHANGES—(i) *In the Alimentary Tract—The Intestines*.—The chief changes found have been inflammation and ulceration (Kinnier Wilson (1914), and Bigland (1920)), though Drummond (1913), in a very complete fatal case in Durban, found the only characteristic post-mortem lesion a thinned bowel without ulceration. Bigland found no thinning of the intestinal wall in any of his cases. A heightened putrefactive process has been said to be indicated by the presence of abnormal amounts of indol and skatol (Sullivan, Stanton, and Dawson, 1921). Enright (1920) states that their significance is apt to be exaggerated, and Hunter, Givens, and Lewis (1916) have shown that pellagra can exist without formation of these substances. Enright considers indol and skatol, if excessive in amount, an index of abnormal protein decomposition. The faecal bacteria, according to McNeal (1913), are abnormal in quantity and variety, 693 new strains being isolated by him from the intestinal contents. Enright (1920) found *Entamoeba histolytica* and the dysentery bacilli, but no abnormal organism which could be proved to have any specific relationship with pellagra.

The Liver.—Atrophy and pigmentation have been described by Kinnier Wilson (1914), fatty degeneration by Bigland (1920) and by Crutchfield (1928). Fibrosis and areas of progressive degeneration and fatty infiltration were present in Susman's cases (1930).

(ii) *In the Skin*.—Hyperæmia, serous transudation, changes in the Malpighian layer, epithelial thickening, and pigmentary deposit were observed by Kinnier Wilson (1914). Stannus (1911, 1913) called attention to a cracked and thickened condition of the epithelium at the corners of the mouth, which he likened to the rhagades of syphilis, and considered a valuable diagnostic sign. Crutchfield (1928) states that neither the skin nor mucous membranes show specific changes pathognomonic of pellagra. He does not agree with the view of many workers that the sun's rays are the only factor in causing the typical distribution of the rash. Stannus (1913), for instance, believed that the sensitisation of the skin to the sun's rays might be due to a circulating toxin like hæmato-porphyrin or phyto-porphyrin. In Crutchfield's opinion, trauma plays a more important part in the production of the skin lesions than photodynamic action. Shelley (1930) also states that sunlight is unnecessary for the production of dermatitis, since it occurs in patients who are bedridden, and who have not been exposed to direct solar rays for months. Shelley believes that it is a trophic phenomenon.

(iii) *In the Nervous System*.—The result of the extensive investigations of many workers on the changes in the nervous system in pellagra may be summarised in the words of Stannus (1927), describing the changes found by Kinnier Wilson (1914). "They are those of a chronic pseudo-systematised degeneration affecting all portions of the peripheral and central nervous system, and resembling somewhat the condition found in subacute combined degeneration of the cord, with no evidence of a vascular or meningeal reaction to infection. According to Winkelman (1926) also the essential nervous lesions of human pellagra consist of a primary degeneration of nerve cells throughout the brain and spinal cord, with the deposition in them of large quantities of a fatty substance, and a hyaline degeneration of the blood vessels with the deposition around them of the same fatty material.

Kozowsky (1912) described the occurrence in the nerve cells of a similar yellow pigment which he considered a constant and general finding in pellagra.

In 1928 Denton stated that chromatolytic change was the only constant lesion in both acute human pellagra and "black-tongue" in dogs.

The majority of workers, including Singer and Pollock (1913), Nicholls (1913), Kinnier

Wilson (1914), and Stannus (1912), have considered that these nervous changes show evidence of being produced by an intoxication.

Stannus, in his earlier work, speaking from clinical evidence, stated: "Taking into consideration the diffuse nature of the cord lesions and the absence of systematisation, it would seem probable that the nervous lesions are produced by a toxin entering the theca by the lymphatic spaces of the posterior roots," while Wilson, on the basis of his pathological investigations, remarked: "It is probable that an ascending toxic lymph stream invades the cord *via* the lumbar and dorsal posterior roots, and that the effects of the toxæmia appear either simultaneously in the nerves and cord or previously in the former."

Stannus's later observation (1927) that "in pellagra, as in beri-beri, it is believed that in the absence of some vital element a toxin may develop and produce the pathological changes in the body," has an interesting bearing upon McCarrison's recent conclusions on beri-beri quoted on page 241.

(iv) *In the Thyroid*.—The toxic theory receives further support from the recent investigations of Susman (1930) into the pathological condition of the thyroid. He emphasises the fact that in every case the thyroid was abnormal. Proliferation of the vesicular epithelium, fibrosis and pigmentation were practically constant findings. "That the glandular function had been disturbed may be surmised because of the presence of the intercellular pigment, and an altered appearance in the colloid, but the chief feature was that some factor, toxic or organismal, or both, had given rise to degenerative changes and replacement fibrosis, on the one hand, and proliferation on the other hand."

(v) *In the Blood*.—The morphology of the blood in pellagra has been investigated by Machwiladse (1929). The chief changes found were a decrease in the number of erythrocytes, leucocytes, and platelets, with a relative increase of lymphocytes and a moderate eosinophilia, when intestinal parasites were present, or when the general health of the patient improved.

(c) PELLAGRA AND ALCOHOLISM.—Many of the early workers in pellagra called attention to the part played by alcohol in the disease. An investigation of 100 cases by Klauder and Winkelman (1928) showed that all except 3 were chronic alcoholic addicts. The cases were divided into 3 groups according to the clinical severity, the first group showing only a typical pellagic dermatitis, whilst the last group were acute fulminating cases, often diagnosed on admission as delirium tremens, the pellagic symptoms developing suddenly after a prolonged debauch and very often terminating fatally. These workers conclude that chronic alcoholics are potential pellagrins, being more susceptible to pellagra, following an insufficient diet, than those not addicted to alcohol. They also state that alcohol is apparently not such an important predisposing cause of pellagra in the southern as in the northern states, other factors that lead to dietary insufficiency being probably less operative in the north than in the south of the United States.

(B) FURTHER PHYSIOLOGICAL ASPECTS OF VITAMIN B₂ DEFICIENCY—(1) *In Rats*.—(a) LACK OF GROWTH.—That the thermostable factor of the vitamin B complex is as necessary for growth as the thermolabile has been shown by the researches of Chick and Roscoe (1928), Findlay (1928), Sherman and Sandels (1929), and others. In Findlay's experiments the average increase in the body-weight of rats fed on a diet deficient in vitamin B₂, but adequate in vitamin B₁, was 3.7 grms. at the end of the experiment, as compared with an increase of 22 grms. on a diet containing both vitamins B₁ and B₂, and a loss of 4.4 grms. on a diet adequate in B₂ but deficient in B₁. The degree of inanition was not so invariable in deficiency of vitamin B₂ as of vitamin B₁.

Chick and Roscoe give as a standard for vitamin B₂ assay a minimum dose which gives an average weekly increase of weight of 10 to 12 grms.

(b) SKIN LESIONS.—The changes in the skin of rats fed on a diet deficient in vitamin B₂ have been considered by the workers who have described them as sufficiently like those in human pellagra to justify the statement that pellagra has been experimentally produced in rats.

Chick and Roscoe (1928) found the skin lesions to develop in young rats after the fifth to sixth week on a diet devoid of vitamin B₂. In their experiments a more constant development of symptoms resulted when the commercial casein in the basal diet was purified by extraction with acid alcohol, showing that commercial casein contains vitamin B₂. The typical skin lesions consisted of dermatitis of the tips of the ears and digits of the paws, and loss of hair from the eyelids, head, nose, mouth, and abdomen.

These lesions were observed also by Findlay (1928), who has described them in detail. The earliest changes (after 6 weeks) appeared on the front of the chest and neck and on the abdomen, later on the legs and paws, and finally over the lower jaws: "At first the skin was red and a little swollen, the hair on the chest wet and bedraggled. After a few days the hair began to fall out and the red tint of the skin was lost as desquamation set in. At first this desquamation was intense, but if the rat lived the skin became smooth, dry, and hairless. The marginal definition of the affected areas was very definite, and bilaterally symmetrical. Occasionally small vesicles were present on the dorsum of the forepaws: these vesicles eventually ruptured, leaving tiny raw areas. In certain rats the hair was fully retained until desquamation, when large flakes of dead skin with hair still adherent were shed, leaving smooth, dry atrophic skin beneath. In some rats also the eyelids became swollen and adherent through the exudation of a blood-stained fluid. The conjunctival lining of the eyelids was congested, but there was no keratitis as in vitamin A deficiency. Later, the hair immediately around the eyes fell out, giving the rats a curious spectacled appearance.

"The tongue in the majority of cases had the appearance of raw beef, especially at the tip and round the margins, while the buccal mucous membrane occasionally showed small ulcerations. Although the majority of rats fed on the diet deficient in vitamin B₂ developed these characteristics, yet there were some which, though placed under identical conditions, failed to develop any symptoms even after 15 to 20 weeks, and in which growth, though much slower than in the control rats receiving vitamins B₁ and B₂, nevertheless continued." No explanation is as yet forthcoming for this phenomenon, but since the stools were normal in colour and consistence, it cannot have been due to refectation.

In less severe cases the rats showed partial recovery, though remaining bald, and suffering from recurrent attacks of conjunctivitis.

In the experiments of Sherman and Sandels (1929) the skin lesions were less pronounced with a less complete deprivation and longer course of vitamin B₂ deficiency. In the less rapidly fatal cases the fur became dry and readily pulled out. A definite saddle-like pattern frequently appeared on the fur of the back. An examination of the area affected sometimes revealed small dry scales on the skin which later might be replaced by larger yellowish crusts on the sides of the back, shoulders, and chest. The skin lesions usually showed a tendency to symmetry. Some of the animals showing marked symptoms of the B₂ deficiency were cured by the sole addition to the diet of substances rich in the B₂ vitamin.

The skin lesions, as described by Thatcher, Sure, and Walker (1930), were bilateral on the head, forepaws, shoulders, jaws, and around the eyes. Histologically, there was necrosis of the skin, with acute inflammation sometimes extending into the muscle. Hyperkeratosis was usually present on each side of the skin ulcer, and where the epithelium had not completely disappeared over the site of the lesion.

(c) LESIONS OF THE NERVOUS SYSTEM.—Like the skin lesions, the nervous lesions found in rats fed on a diet lacking in vitamin B₂ resemble those found in human pellagra. In the

experiments of Stern and Findlay (1929) the changes consisted of swelling and vacuolation of the anterior horn cells of the spinal cord with deposition in them of lipochrome pigment and great increase in number of π granules in peripheral nerves. The presence of granules in nerves is not a lesion peculiar to pellagra, but shows that there is some alteration in myelin metabolism.

The amount of pigment present was directly proportional to the length of time that the animal had been on the diet, and it was absent in those rats which had been killed during the first acute attack of dermatitis. Normal rats of the same age did not show any lipochrome pigment in the nerve cells.

Mental Changes.—Findlay (1928) noticed a psychological change in rats deprived of vitamin B₂. Animals which had previously been tame and docile, having been handled from birth, quite suddenly became irritable and suspicious, biting at the least provocation. Findlay points out that in man the onset of pellagra is often associated with a change in character, sufferers from the disease becoming fractious and suspicious.

(d) *LOSS OF APPETITE.*—The food intake was gradually reduced, and during the last few days of life was small, but there was no evidence of the complete loss of appetite characteristic of vitamin B₁ deficiency.

(e) *FALL OF BODY TEMPERATURE.*—The body temperature became subnormal when the animal became moribund.

(f) *CHANGES IN ENDOCRINE ORGANS.*—The spleen, testicles, and thymus showed atrophy while the adrenals had increased in weight.

(i) *The Adrenals.*—The increase in weight of the adrenals was not a terminal phenomenon since it was found in rats shortly after the appearance of the cutaneous lesions at a time when the daily food intake, although reduced, had not yet fallen to minimal amounts, and when inanition, as judged by the weight of the thymus and spleen, was not advanced. In order to prove that the adrenal hypertrophy was not due to a deficiency of vitamin B₁, two rats which were showing early signs of vitamin B₂ deficiency were given daily doses of 1.5 c.c. of a yeast extract containing vitamin B₁. After ten days they were killed when the adrenals were found to be still enlarged in spite of the large doses of vitamin B₁. The histological changes were similar to those in vitamin B₁ deficiency.

(ii) *The Testes.*—The testes in some rats deprived of vitamin B₂ showed spermatozoa within the tubules, though spermatogenesis was far from active. There was a tendency for the spermatozoa to fuse together while the tails stained intensely with the hæmatoxylin: the rest of the germinal epithelium was normal. In others there was complete absence of spermatozoa.

(g) *CHANGES IN THE ALIMENTARY SYSTEM*—(i) *Mouth.*—In rats lacking vitamin B₂, Findlay found the buccal mucous membrane congested, and in places there were small ulcerations, and the stratified epithelium of the tongue was also infiltrated with polymorphonuclear leucocytes.

(ii) *Stomach.*—Findlay describes a papillomatous change in the cardiac portion of the stomach which in the rat is lined by squamous epithelium. This lesion was similar to that described by Fujimaki and co-workers (1927) as a result of vitamin A deficiency. Findlay suggests that the explanation advanced by Pappenheimer and Larimore (1924) may be correct, namely, that the condition is due to the ingestion of hair, which is shed in large amounts by rats lacking vitamin B₂.

(iii) *Intestines.*—Congestion of the submucosal vessels and, in moribund animals, atrophy of the lymphoid tissue and muscular coats were the chief changes found.

(2) *In Dogs.*—In 1928 Goldberger and Wheeler observed a pathological condition in dogs fed upon—(1) a diet known to cause spontaneous pellagra in man; and (2) upon a diet which

experimentally has given rise to pellagra in man. This condition, they stated, "commences with a stomatitis followed by necrosis, and presents features in no way distinguishable from the disease known as black-tongue which occurs naturally in dogs."

A complete investigation of the changes in experimental black-tongue was made by Denton (1928) who stated that the lesions "originate in a degenerative process affecting the superficial connective tissue of the mucous and dermal membranes." Secondary changes follow in the overlying epithelium, and the final picture is one of an intensely necrotic and diphtheritic inflammation of the upper alimentary tract. No histological changes were found in the central nervous system or in any other organs.

The macroscopic appearances found post-mortem were as follows :

(a) **IN THE MOUTH.**—All grades of inflammation, varying from a reddening of the oral mucosa in early stages to superficial necrosis, with the formation of a pseudo-membrane, as the disease neared its termination. This latter condition was apt to spread downwards involving the whole upper alimentary tract.

(b) **SKIN.**—Four out of seven male dogs showed lesions of the scrotal skin.

(c) **INTESTINES.**—Earlier cases showed no changes in this situation, but in three later specimens the colon was thinner than normal, and the mucous membrane was reddened and stained with reddish-brown mucus.

(d) **OTHER ORGANS.**—No gross changes were found in the bones, periosteum, brain, or cord.

In comparing the lesions of experimental black-tongue with those of human pellagra, Denton states : "The lesions of the skin, mouth, pharynx, œsophagus, and colon in pellagra and in experimental black-tongue in dogs, show very similar gross appearances. Histologically, the lesions of both appear to have their inception in a degenerative process in an analogous tissue element. The processes of repair in both result in fibrotic replacement and in pathological vascularisation of the superficial stroma of the mucous membrane of the upper alimentary tract and of the corium." Secondary infection tends to occur.

Fatty Degeneration of the Liver and Kidneys.—This condition was observed by Sebrell (1929) in the course of experiments on the black-tongue preventive in certain foods. The most striking change was in the liver where, in some cases, most of the normal tissue appeared to have been replaced by fat. Microscopic examination showed fatty degeneration of the liver cells, heart muscle, and kidney tubules. The spleen was atrophied. Sebrell believes the condition to be associated with some peculiar feature of the diet, and not necessarily in direct relationship to black-tongue.

LVIII. VITAMIN B₂ IN FOODSTUFFS.

Although the distribution of vitamin B₂ follows and coincides more or less strictly with that of vitamin B₁, the relative values of certain foodstuffs differ with regard to each factor. Particularly is this the case with cereals, milk, and beef. Meat and milk are much richer in vitamin B₂ than B₁, while cereals are a better source of B₁ than B₂. Both are present in yeast, but the relative amounts differ according to the source.

(A) **YEAST.**—According to Randoin and Lecoq (1926), yeast extracts from different sources never have the same vitamin B₁ and B₂ value. They found, for example, that an extract from beer yeast contained both the growth-promoting and the antineuritic factor, while an extract from distillery yeast contained the growth-promoting but not the antineuritic.

Brewers' yeast has also been stated by Bloxsom (1929) to be a good source of the growth-promoting factor. In both premature and normal infants he observed a marked increase in the rate of gain and growth when brewers' yeast was added to the diet. Dried brewers' yeast has given a certain amount of success in the treatment of pellagra by Cenley (1930).

The two factors can be separated either by autoclaving, by treatment with alkali, or by adsorption on fuller's earth. The first two processes destroy the antineuritic factor, and the third removes it.

According to Hassan and Drummond (1927), normal growth follows the addition to a diet inadequate in growth-promoting content of yeast extract (marmite) which has been treated with hot alkali. The supplements which Kennedy and Palmer (1928) have found successful in maintaining growth are 0.2 gram daily doses of yeast which has been completely extracted with hot 85 per cent. alcohol, the residue of yeast left after the removal of the Osborne and Wakeman fractions, or yeast that is autoclaved to destroy its recognised growth-promoting properties.

(B) **CEREALS**.—(1) **Wheat and maize** are comparatively poor in vitamin B₂. Goldberger and co-workers (1928) found that whole wheat contained the black-tongue preventive (which they consider identical with the pellagra preventive), but in small amounts. Goldberger and Wheeler (1927) had already found that commercial wheat germ in a dosage of 5 ounces per day gave fairly satisfactory results as a pellagra preventive. Hunt (1928) also found wheat and maize poor sources of vitamin B₂.

On a diet containing 15 per cent. of wheat supplemented with 0.4 gram of autoclaved yeast, fair growth occurred, but the rats did not reproduce. On increasing the wheat to 25 to 35 per cent., growth and reproduction occurred.

Wheat and maize have been tested for the vitamin B₂ content of their different constituents by Aykroyd and Roscoe (1929). They found that 50 per cent. of whole wheat was required in the diet for sufficient growth, and that the endosperm was a poor source of vitamin B₂. Wheat embryo and bran were equivalent, 15 to 30 per cent. providing sufficient vitamin B₂. The value of maize as a whole was lower than that of wheat, 50 per cent. of maize being insufficient for normal growth. The following constituents are placed in descending order as regards their vitamin B₂ content (estimated in dry weight):

1. { Wheat germ.
 { Wheat bran.
2. Wheat pollard.
3. Whole wheat (Manitoba).
4. Whole wheat (English).
5. Whole maize (white).
6. Whole maize (yellow).
7. Maize germ meal.
8. Wheat endosperm.
9. Maize endosperm.

(2) **Corn-starch** is stated by Evans and Burr (1928) to be a source of the growth-promoting factor.

(3) **Flour**.—According to Hartwell and Mottram (1929), wholemeal flour contains approximately only the same amount of vitamin B₂ as white flour.

(C) **MILK**.—(1) **Fresh whole milk** has been found by Goldberger and co-workers, and also by Aykroyd and Roscoe, a satisfactory preventive of both pellagra and black-tongue.

It has been stated by Macy, Outhouse, and Graham (1927), however, that human milk is not a good source of the growth-promoting factor. Normal growth in rats was only secured when human milk was given in progressively increasing amounts of 25 to 35 c.c. daily. Hunt and Krauss (1928) found milk from cows on winter feed rich in vitamin B₂ as compared with vitamin B₁, while Sherman and Axtmayer (1927) also showed that milk is relatively richer in vitamin B₂.

(2) **Malted milk** is stated by Quinn and Brabec (1930) to be rich in vitamin B₂.

(3) **Casein** has been shown by Chick and Roscoe's (1928) experiments to contain a certain amount of vitamin B₂. They obtained anomalous results until they purified with alcohol extraction the commercial casein which formed part of the basal diet. Vitamin B₂ has also been shown by Evans and Burr (1928) to be present in commercial casein, and in casein leached by treating with dilute glacial acetic acid by Goldberger and co-workers (1930).

(D) **ANIMAL TISSUES.**—(1) **Beef.**—Lean beef has been shown to be preventive for both pellagra and black-tongue by Goldberger (1928) and by Aykroyd and Roscoe (1929), and the latter find it a better source of vitamin B₂ than cereals.

Hoagland (1929) finds lean beef a less valuable source of vitamin B₂ than lean pork.

(2) **Salt pork** is stated by Goldberger and co-workers to be poor as a black-tongue preventive.

(3) **Liver.**—Aykroyd and Roscoe (1929) put dried ox liver at the head of their list of vitamin B₂-containing foods, and Goldberger and co-workers (1928) state that pork liver contains black-tongue preventive, but has not yet been tested for its value in pellagra.

It is interesting to note that Mollow (1928) reports good effects from treating two long-standing cases of pellagra with liver, and suggests that, like pernicious anæmia, pellagra may be the result of an intestinal intoxication.

(4) **Fish.**—(a) **CANNED HADDOCK** (Goldberger and co-workers (1930)) and

(b) **CANNED SALMON** (Goldberger (1928) and Goldberger and Wheeler (1929)) contain the black-tongue preventive, and on the basis of experiments on dogs they recommend that the latter be used in the prevention and treatment of human pellagra.

(c) **COD-LIVER OIL** is preventive both for black-tongue and pellagra.

(5) **Eggs.**—Egg-yolk is stated by Aykroyd and Roscoe to be a better source of vitamin B₂ than cereals. Goldberger has found it preventive against black-tongue.

(E) **VEGETABLES AND FRUITS.**—(1) **Dried legumes**, such as peas, are, according to Aykroyd and Roscoe, rather low in vitamin B₂ content, but higher than whole wheat and maize.

Cowpeas, according to Goldberger and Wheeler (1927), contain relatively small quantities, 5 ounces a day not being protective against pellagra. Dried peas also contain a small amount (Goldberger and co-workers (1930)).

(2) **Carrots and Turnips (rutabaga).**—Goldberger and Wheeler (1927) state that if these vegetables possess pellagra-preventive action it must be rather feeble, since a daily supplement of cooked rutabagas, equal in each case to 453 grms. of the dressed raw vegetable failed. He suggests, however, that the quantity eaten, although apparently liberal, was too small.

(3) **Spinach** is stated by Eddy (1927) to be richer in vitamin B₂ than B₁ and spinach, lettuce, and cabbage are stated by Roscoe (1930) to be valuable sources of vitamin B₂.

(4) **Fruits.**—(a) **CANNED TOMATOES** possess a pellagra-preventive potency, though of a rather feeble order. Goldberger and Wheeler found that a daily quantity of the expressed juice of approximately 1200 grms. had a well-marked action.

(b) **BANANA** is stated by Eddy (1927) to contain at least three times as much vitamin B₂ as B₁.

LIX. VITAMIN C.

It was suggested by Drummond in 1918 that the term "water-soluble C" should be applied to the accessory food substance whose connection with the development of scurvy had been proved by many investigators.

During the last few years there have been several attempts to prove that vitamin C is composed of two distinct factors. The evidence brought forward by Scotti Foglieni (1926 and 1927) cannot be regarded as conclusive since the chemical reaction upon which it is based

is not accepted by the majority of workers (including Zilva (1925)) as characteristic of vitamin C. Scotti Foglieni claimed to have fractionated the vitamin from fresh lemon juice into a true antiscorbutic factor (obtained by distillation) and another fraction which gave the blue coloration with phosphomolybdotungstic acid described by Bezssonoff (1922 and 1926) as specific for vitamin C. Zilva (1925), however, tested a great number of antiscorbutic substances with this reagent, and found that while some gave the reaction, others did not. On the other hand, some substances which had no antiscorbutic potency gave an intense coloration. Zilva himself (1928) postulates two factors in the vitamin C complex which he provisionally designates the "thermolabile factor" and the "reducing agency" respectively, but he does not make the positive assumption that these substances are actually preformed in the cell. He suggested in 1927 that the reducing factor might act as a protective agency for the antiscorbutic.

Further investigation (1928) showed that when the reducing properties were destroyed (as, for instance, during the process of chemical fractionation) the antiscorbutic potency of a concentrated preparation containing vitamin C deteriorated more rapidly. From this point of view he suggests that the formation of vitamin C may be a complex mechanism in which the antiscorbutic factor, which can withstand the drastic treatment of heating at 143° C. in the absence of air, becomes labile in the presence of air when the protective conditions of the medium are removed.

Bezssonoff has also made experiments to prove the duality of vitamin C. In 1926 he observed that as much as 180 mgrms. of a substance from fruit juice was not effective as an antiscorbutic when administered alone, but that 40 mgrms. of the same substance became potent when given with 60 c.c. of heated milk. He concluded, therefore, that vitamin C consisted of two substances which he called C_1 and C_2 . In later experiments (1928) he obtained a substance from cabbage juice by treatment with neutral lead acetate, filtration, concentration of the filtrate, addition of excess of $Ca CO_3$, and precipitation with excess of alcohol at 95° C. which did not contain the factor he had called C_2 . Only by the addition of heated milk or lemon juice to this product could protection from scurvy be obtained in the guinea-pig. An attempt to isolate the factor C_2 was made by neutralizing partially (pH 4.5) the original lead acetate filtrate and then precipitating with alcohol. The procedure was not entirely successful.

From the biological aspect, Randoin and Lecoq (1927) have claimed the separation of vitamin C into a true antiscorbutic factor and another which is concerned in the metabolism of iron and the formation of hæmoglobin. They state that it is possible to produce in young rabbits on a scorbutic diet, to which autoclaved lemon juice has been added, a chronic state of anæmia without symptoms of true scurvy. Their experiments have not, however, been substantiated.

(A) SOURCE OF VITAMIN C.—The antiscorbutic factor is found in nature when living processes are actually taking place. Thus, in contradistinction to the antineuritic vitamin B, which is found chiefly in dry seeds, vitamin C is produced during the germination of seeds. This process was demonstrated by Holst (1907) in barley. Fürst (1912) also found that cereals and pulses which have no antiscorbutic potency become potent if soaked in water and allowed to begin germination for 2 or 3 days. In the case of pulses this result has been confirmed by Chick and Hume (1917), Chick and Delf (1919), and clinically by Wiltshire (1918), who succeeded in curing 27 cases of mild scurvy by including in the dietary 4 ounces of germinated haricot beans.

In the case of cereals the production of vitamin C during the germination was doubted by Weill, Mouriquand, and Peronnet (1918), but it has since been confirmed by several workers, including Harden and Zilva (1925), Kucera (1928), Simonik (1929), etc.

Harden and Zilva showed that antiscorbutic activity was acquired by barley even before germination is actually observed. This was demonstrated by a malting experiment, in which the grain was tested in the various stages of the process. In the preparation of malt, the barley is first steeped. It is then allowed to germinate at a suitable temperature, after which it is kilned to arrest the germination. After removing the sprouted germ, malt is obtained.

Harden and Zilva found that after steeping, and before any visible signs of germination appeared, the barley, which was previously inactive, showed marked signs of antiscorbutic potency. So also did the germinated barley; its activity was, however, destroyed in the kilning.

Kucera investigated the development of vitamin C in several varieties of cereal by feeding the seeds, after germination for varying periods, to guinea-pigs.

With rye, the seeds were able to prevent scurvy after germination for 24 hours, but were not effective if germinated for a shorter time. With oats, the development of vitamin C appeared to be slower, and seeds did not protect from scurvy until they had been germinated for 4 days.

Similar experiments on the vetch, bean, and pea, by Simonik showed that vitamin C could be detected in all three grains after soaking for 6 hours, the minimum dose then being 20 grms. The vitamin C content increased rapidly during germination, the minimum dose after 20 days' germination being 0.75 gram.

The effect of allowing the seeds to soak in dilute acid instead of water has been observed by Luettmerding (1929) to accelerate the production of vitamin C. The seeds of wheat, oats, and peas were allowed to soak for varying lengths of time in (a) distilled water, (b) 0.05 per cent. citric acid, and (c) 0.1 per cent. citric acid. The seeds were then fed (20 to 30 grms.) to guinea-pigs on a diet otherwise free from vitamin C. With wheat, the grains soaked in water did not contain sufficient vitamin C to maintain life until they had been soaked for 15 hours. The grains of wheat soaked in 0.05 and 0.1 per cent. citric acid contained a sufficient amount of vitamin C after 12 hours' and 6 hours' soaking respectively. With oats, the grains soaked in water for 48 hours did not contain enough vitamin C to maintain life, while the grains soaked in 0.05 and 0.1 per cent. citric acid were capable of maintaining life after 48 hours' and 24 hours' soaking respectively. The development of vitamin C in peas was so rapid that 3 hours' soaking in water sufficed to produce a protective amount. An accelerating action of the acid media was therefore not detected in this case. The effect of light on the synthesis of vitamin C in germinating seeds was studied by Eggleton and Harris (1925). They stated that vitamin C did not depend for its synthesis upon the action of light. Heller (1928), however, states that the amount of vitamin C is greater in seeds germinated in the light than in etiolated seeds.

(B) **CHEMICAL NATURE OF VITAMIN C.**—It is pointed out by Zilva (1925) that the investigation of the chemistry of the antiscorbutic factor is beset with difficulties chiefly on account of its close association in nature with a large bulk of impurity. A very active fraction obtained from lemon juice was shown by Zilva to be not associated with the sugar nor with the bulk of the nitrogenous fraction of the lemon juice. The chief chemical characteristics of this potent fraction were stated by Zilva in 1925 to be that it contained very little nitrogen, merely traces of phosphorus, and that it decolorised potassium permanganate and reduced ammoniacal silver nitrate in the cold. By later modifications, however, Zilva (1927) succeeded in separating the reducing factor from the antiscorbutic. The reducing factor, though destroyed by the same agencies which inactivated the antiscorbutic factor, namely, heat and alkalinity, was found to be destroyed at a different rate. Another fact which showed that the reducing capacity was not directly associated with the antiscorbutic activity was the separation of fractions which were inactive from the point of view of preventing scurvy, but which still

had a high reducing capacity. Zilva observed also that upon removal of the reducing agent the antiscorbutic factor, though not immediately lost, disappeared very rapidly. He suggested that the stability of the antiscorbutic factor depends upon a chain of reactions which are in equilibrium in the living cell.

In the purest fractions, iodine was present (Daubney and Zilva (1926)) in a compound of colloidal or semi-colloidal nature and separable from the active principle by diffusion.

Hoyle and Zilva (1927) found that a concentrated preparation contained traces of iron, phosphorus, and sulphur, but they were not able to prove that these elements were included in the structure of the vitamin. Using a method of differential dialysis (see below), Zilva came to the conclusion that the size of the active molecule of the antiscorbutic factor was approximately the same as that of a hexose.

Later investigations (1928) have somewhat modified his view. The differentiation of vitamin C into the antiscorbutic factor and the reducing agent, with the supposition of a protective quality of the latter, have led him to suggest the possibility that vitamin C may consist of a large active molecule which, on destruction of the tissues of the plant, may become degraded or modified, with a tendency towards greater instability. His observations on dialysis also, with the discovery that the reducing agency dialyses under the same conditions as those previously recorded for vitamin C as a whole, suggests to Zilva the possibility that the antiscorbutic factor may have been inactivated and not actually diffused out. In this case the molecule may be larger than he at first surmised.

Bezssonoff (1922) suggests that the antiscorbutic factor contains an unstable radical which can be split off in the form of quinol.

(C) **CONCENTRATION OF VITAMIN C.**—Isolation of the antiscorbutic factor has been made particularly difficult by its instability.

(1) **Methods of Zilva.**—Zilva (1922–1928) has devised various methods for overcoming the difficulty.

(a) **REMOVAL OF SOLIDS.**—The elimination of citric and other acids from lemon juice was carried out by the addition of calcium carbonate, and the neutralised solution was treated with excess of absolute alcohol which removed other substances such as proteins without destroying the antiscorbutic activity (Harden and Zilva (1918)). By this means about 85 per cent. of the solids of the juice were removed, leaving a neutral residue which, owing to its low solid matter content, could be concentrated many times, yielding a highly active preparation.

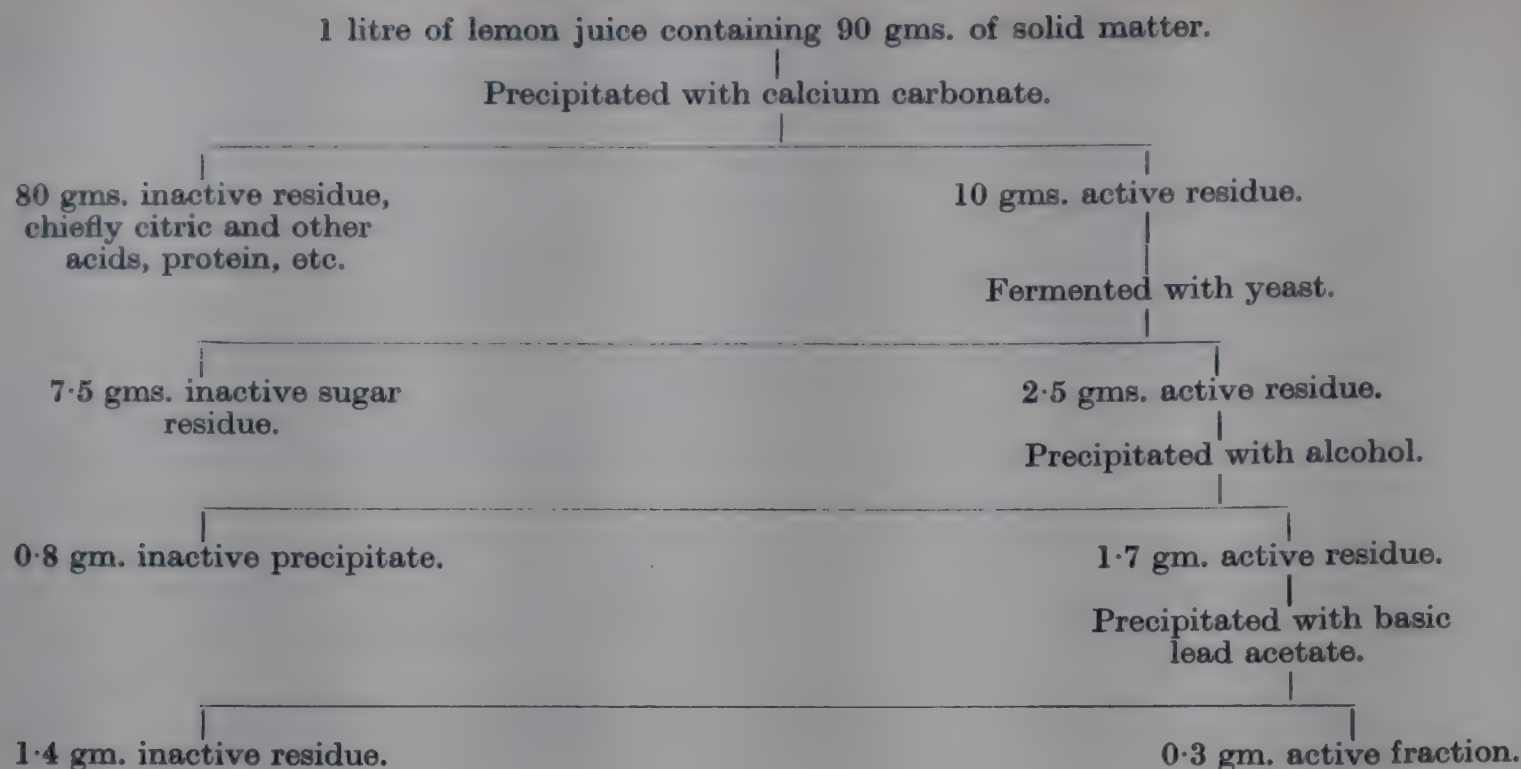
(b) **REMOVAL OF SUGAR.**—This neutral active residue was found to consist mainly of invert sugar. The ordinary chemical means of removing the sugar would have inactivated the antiscorbutic factor. Zilva therefore employed fermentation with yeast as a method of removal, the total activity remaining in the residual fraction.

(c) **PRECIPITATION OF ACTIVE FACTOR.**—Basic lead acetate was found to precipitate the entire vitamin content, and by removal of the lead by treatment with acetic acid and magnesium sulphate a very active fraction was obtained. The solids of such an active solution form only about 0.03 per cent. of the solution; the activity is the same as that of the original lemon juice which contained 9 per cent. of solid matter.

The table at top of next page shows the scheme of fractionation.

(d) **ALTERNATIVE METHOD OF ZILVA (1925).**—Decitrated lemon juice, concentrated to two-fifths of its volume, is precipitated with basic lead acetate without previously fermenting the juice. After centrifugalisation the supernatant liquid is again treated with basic lead acetate. Removal of the lead, and further precipitation with alcohol, leaves a fraction which is not so pure as the one previously discussed.

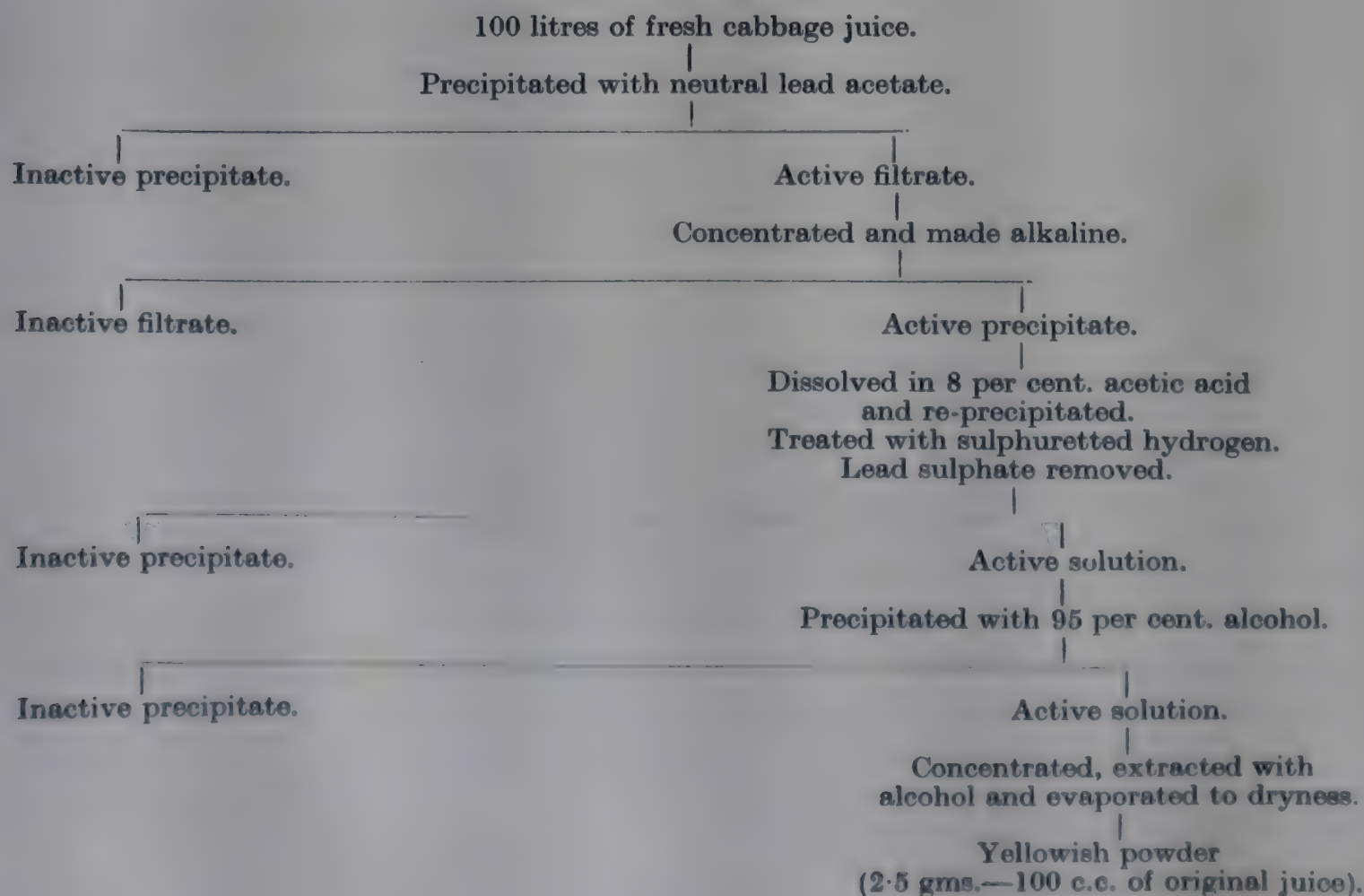
Zilva (1927) mentions that most of the antiscorbutic factor in lemon juice is precipitated



by lead acetate within the *pH* range of 5.4 to 7.2, the greater part being precipitated in the fraction which comes down at *pH* 7.0. The use of excess of basic lead acetate is undesirable, since there is a risk of inactivation in the resulting alkaline solution.

(2) **Method of Bezssonoff** (1922, 1923, 1925).—Bezssonoff has taken advantage of the fact that neutral lead acetate does not precipitate the antiscorbutic factor while basic lead acetate does, and has combined the two reactions in the successive steps of isolation.

Neutral lead acetate, according to Vedder and Lawson (1927), removes from the active extract the bulk of the phosphorus and sulphur without impairing the curative properties. The following table shows Bezssonoff's scheme of fractionation :



(D) PHYSICO-CHEMICAL PROPERTIES OF VITAMIN C—(1) Solubility—(a) IN WATER.—Vitamin C is soluble in water.

(b) IN ALCOHOL.—Absolute ethyl alcohol, according to Vedder and Lawson (1927), gives the purest extracts of vitamin C. Absolute acid alcohol and 80 per cent. alcohol containing 1 per cent. of citric acid are also good solvents (Freise (1914); Freudenberg (1914); Hart, Steenbock, and Lepkowsky (1922), and others). It is insoluble in butyl alcohol (Hart, Steenbock, and Lepkowsky).

(c) IN ACETONE, ETHER, BENZENE, CHLOROFORM, ETC.—Vitamin C is insoluble in these reagents according to Hart, Steenbock, and Lepkowsky (1922); Holst and Frölich (1907); Vedder and Lawson (1927). (It is stated by Rathéry and Kourilsky (1928) that acetone inactivates the vitamin.)

(2) Adsorption.—Unlike the antineuritic vitamin B, the antiscorbutic factor is not adsorbed by fuller's earth from neutral solution (Harden and Zilva (1918); Fraenkel and Hager (1921)). Nor is it taken up by colloidal ferric hydroxide. According to Ellis, Steenbock, and Hart (1921), however, it is taken up from orange juice by blood charcoal.

(3) Dialysis.—Zilva and Miura (1921), using collodion membranes soaked in alcohol of various strengths, have shown that vitamin C dialyses through membranes of 90 per cent. but not 95 per cent. concentration, *i.e.* through membranes which permit the passage of substances of such molecular dimensions as methylene-blue, neutral red, and safranine.

They found also that the sugar and nitrogenous substances of lemon juice diffused at a different rate from the antiscorbutic factor, confirming their opinion already formed during attempts to concentrate the factor, that vitamin C was not associated with either the sugar or the nitrogen. In later experiments (1928) Zilva discovered that the reducing agency of the vitamin C complex dialysed under the same conditions as those which he had previously believed specific for the antiscorbutic factor. This discovery has left him uncertain as to whether the antiscorbutic factor had actually diffused or had been inactivated by the removal of the protective reducing agency.

It is pointed out by Hoyle and Zilva (1927) that the phosphorus, sulphur, and iron in the active fraction of concentrated lemon juice behave on dialysis like the active fraction itself, passing but slightly through collodion membranes soaked in 88 per cent. alcohol but passing completely through collodion membranes with a 95 per cent. alcohol index.

They suggest, however, that a connection between them and the antiscorbutic activity must be postulated with caution. If there be any connection these elements must be either in physical or chemical association with some other substance or must themselves be in a definite physical state responsible for their physiological function.

(E) STABILITY OF VITAMIN C—The stability of vitamin C may be said to be generally less than that of other vitamins, especially with regard to methods of preservation, its extreme susceptibility to oxidation rendering it difficult to preserve.

(1) Factors in Destruction of Vitamin C—(a) OXIDATION.—The susceptibility of vitamin C to oxidation is so great that it has come to be regarded as a general rule that foods which are powerfully antiscorbutic in the fresh state lose practically the whole of their potency by exposure to air. It has been stated, however, by Lepkowsky, Hart, Hastings, and Frazier (1925) that this oxidative destruction can be prevented to a great extent by protecting them with a layer of paraffin oil. Zilva (1922) found that while no destruction of the vitamin C in decitrated lemon juice occurred on heating in an atmosphere of carbon dioxide, its antiscorbutic value was practically entirely destroyed either by exposure to ozone or simply by aspirating air through the juice for 12 hours at room temperature.

Delf (1918, 1920), also, in the course of her experiments on the effect of heat on vitamin C,

found it increasingly apparent that heat is of importance only in so far as it accelerates oxidation, and these results have been amply confirmed by other workers.

Dutcher, Harshaw, and Hall (1921), for instance, carried out experiments with orange juice, and found that heating for 30 minutes at 63° C. in closed vessels had little effect upon the antiscorbutic value, but that heating to the same temperature in the presence of hydrogen peroxide resulted in marked vitamin destruction.

Hess and Unger (1921) also found that when 4 c.c. of a normal salt solution of hydrogen peroxide were added to a litre of raw milk, incubated overnight, results similar to those of feeding experiments with milk which had been autoclaved for 1 hour at 120° C. were obtained. The same workers, at a later date, found that the presence of minute quantities of copper in milk—1.4 parts to a million, which resulted from heating milk in copper vessels—greatly accelerated the destruction of the antiscorbutic vitamin.

Orange juice subjected to oxygen for a short period was likewise found to have lost some of its potency, and milk or tomato juice which had been shaken had lost some of its vitamin C. An interesting but unsuccessful attempt was made by Zilva and Daubney to reactivate the oxidised vitamin C by suitable reduction methods—hydrogenation in the presence of platinum black and by electrolysis. No regeneration of antiscorbutic activity could be demonstrated by feeding experiments after the active principle had once been rendered inert by the preliminary oxidation.

(b) HEAT.—The destruction of vitamin C by heat depends upon the presence of other factors, of which oxidation is of chief importance; in fact, it has been debated in experimental circles whether heat *per se* is destructive at all, or whether it merely speeds up oxidation, which is the true destructive force. Increase of temperature seems to be a less important factor than the time of heating.

The most conclusive experiments have been carried out by Delf (1918) upon the effect of heat on the antiscorbutic properties of cabbage, and she states that the antiscorbutic factor was found to be exceedingly sensitive to a temperature below 100° C., about 70 per cent. of the original value being lost after 1 hour's heating at 60°, and over 90 per cent. after the same period at 90°. Twenty minutes' heating at 90° or 100° was estimated to cause destruction about equal to that of 60 minutes at 60°. In the case of the fat-soluble factor of cabbage, the destruction was much less rapid; 1 or 2 hours at temperatures of 100° to 120° only causing slight destruction, 2 hours at 130° being necessary for serious destruction to take place.

The following table shows her results in quantitative form:

Substance.	Treatment.	Time. Min.	Temp. C.	Limiting Protective Ration.	Per cent. Destruc- tion.	Author.
				Grams.		
Green cabbage	Raw	—	—	1	—	Delf, 1918.
	Simmered	60	60	5	80	do.
	Steamed	20	100	>5	80	do.
	”	20	90	5	>80	do.
	”	120	130	<15	—	do.
	Canned (2 weeks old)	60	90-100	>7.5	>70	Campbell and Chick, 1919.
Runner beans	Raw	—	—	5	—	do. do.
	Canned (3 months old)	140	100	>20	>75	do. do.
Germinating lentils	Raw	—	—	5	—	Chick and Delf, 1919.
	Boiled	15	100	12	—	do. do.

or, stating the quantitative result in another way, "by boiling cabbage for 1 hour at 212° F. we get 95 per cent. destruction of vitamin C."

(c) COOKING.—With regard to the effects of cooking on the destruction of vitamin C in foodstuffs, it may be said that simple cooking is not highly destructive, but that prolonged boiling followed by drying is very destructive, especially to vegetables. Very little vitamin C passes into the water in which the vegetables are cooked, and it is noteworthy that at 70° C. the destruction is practically as complete as at 130° C.

Harden and Zilva (1918) have shown that the presence of alkali has a deleterious effect upon vitamin C, but here again there is a definite connection between reaction and oxidation in the destruction of activity. When an antiscorbutic solution is made about $\frac{N}{20}$ alkaline (about pH 12) and allowed to stand for 24 hours in the air, it is totally inactivated. If this change of reaction is brought about whilst taking great precautions in excluding air, and the liquid kept for 24 hours under anaerobic conditions, no loss takes place at all. They have, nevertheless, called attention to the danger involved in the practice of adding sodium carbonate when boiling green vegetables.

The addition of acid, on the other hand, does not minimise the destruction of vitamin C during cooking. Delf (1918) showed that when 0.5 per cent. of citric acid was added to the water in which germinated lentils were boiled, the loss in antiscorbutic property was, if anything, greater than when no addition of acid was made.

An interesting fact with regard to the cooking of apples has emerged from the researches of Bracewell, Hoyle, and Zilva (1930) into the antiscorbutic potency of apples. They found that baking apples in their skins in an oven at about 115° C. for 50 minutes scarcely affected their antiscorbutic potency.

(d) DRYING.—(i) *Vegetables*.—The early experiments of Holst and Frölich (1912) were mainly directed towards finding some method of preserving vegetables without destroying their antiscorbutic property.

They found that dried cabbage retained its efficiency when the material was kept dry, but lost it when it was exposed to water vapour. Later experiments (1920) proved that an efficient drying agent, such as phosphorus pentoxide, preserved the antiscorbutic property of cabbage for 18 to 26 months, while calcium chloride was less efficacious.

Delf and Skelton (1918), however, found a loss of more than 93 per cent. of the antiscorbutic potency when the cabbage was dried at 37° C. and stored at air temperature in a closed bottle for 2 to 3 weeks.

Holst and Fleischer (1927) found that dried cabbage, subjected *in vacuo* for 5 hours, after 1 week's preliminary drying at 37° C. to temperatures of 55° C., 65° C., and 72° C., retained its activity for at least a year. If, after the preliminary drying, it is further dried for some weeks by exposure to phosphorus pentoxide, it will subsequently retain its antiscorbutic potency for more than a year in sealed vessels maintained at a temperature of 34° C.

Dried tomato has been found by Givens and McClugage (1921) to be active in doses of 1 gram.

Tomato, potato, and cabbage, dried in the sun in India, have been shown by Shorten and Roy (1919, 1921) to retain some of their antiscorbutic potency, while carrots, egg-plant, spinach, turnips, and turnip-tops lose practically all of it.

It has been suggested by Mattei (1927) that the death of animals on dried cabbage may be due not to vitamin deficiency, but to the denaturing of vegetable proteins. He states that the vitamin appears to be destroyed when a temperature is reached which causes harmful changes in the proteins.

(ii) *Fruits*.—Dried tamarinds, mango, and cocum were found by Chick, Hume, and Skelton (1919) to possess a low antiscorbutic potency.

Whole dried orange has been stated by Goss (1925) to be equivalent in a dosage of 0.4 gram to 2 grms. of fresh whole orange. Dried Californian peaches have been tested by Morgan and Field (1929). They obtained the somewhat surprising result that drying, combined with the employment of sulphur dioxide, had a much less destructive result than sun-drying or dehydration without sulphur dioxide. The sulphur dioxide was employed in the ratio of 1840 to 1875 parts per million. The antiscorbutic power of sulphured dried peaches was high, 1 gram of the product being equivalent to 1.5 to 3 grms. of oranges or lemon juice or fresh tomato, and to 3 times that quantity of banana, 6 times the amount of raw apple or pear or cooked potato.

The question was raised as to whether the protective action was due to decreased oxidation due to coagulation, or because of the reducing action of sulphur dioxide, or to greater stability of the vitamin in the more acid mediums. Similar experiments are being conducted on prunes and apricots and the effect of lye-dipping on vitamin C content is also being investigated.

(iii) *Fruit Juices*.—Fruit juices, on the other hand, can be dried *in vacuo* without much loss of antiscorbutic potency.

Harden and Robison (1919, 1920) found that dried lemon juice kept in a desiccator for 15 months at room temperature lost only 50 per cent. of its activity, while Basset-Smith (1920) showed that dried lemon juice tablets retained their vitamin for at least a year.

Similarly the vitamin C in dried orange juice was effective, as shown by Gertrude J. Humphrey (1926), after being kept under partial vacuum for 5 years. Harden and Robison (1921) have also confirmed the fact that lemon and orange juice dried *in vacuo* at 35° C., and then kept at ordinary temperature, keeps its activity for two years, though, if kept at 29° C., the same product had lost 85 per cent. after fourteen months.

Desiccated orange juice has been stated by Goss (1925) to be a good source of vitamin C, 0.5 to 1 gram being equivalent as a minimum protective dose to 1.5 to 3 c.c. of fresh juice.

Orange juice dried by the "spray process" is stated by Givens and McClugage (1921) to be still effective after 14 to 20 months. These workers also found commercially dried lemon juice, tomato juice, grape-fruit juice, and orange juice all active after 14 to 20 months, whereas grape juice and raspberry juice were inactive.

(2) *Destruction of Vitamin C in Milk—By Dried-Milk Processes*.—There appears to be some difference of opinion as to the relative destruction of vitamin C in the two chief processes of drying milk—the Just roller process and the spray. An investigation undertaken by Cavanagh, Dutcher, and Hall (1924) was stated to prove that dried milk, evaporated in a continuous steel vacuum evaporator, through which the milk passed in a very short time, lost none of its antiscorbutic potency. The lack of destruction of vitamin C was attributed to the rapidity of the drying process and the comparative absence of moisture. Jephcott (1923), on the other hand, having made a large number of examinations of spray process powders, states that this type of milk powder is very prone to undergo oxidative deterioration, since the fine spray is forced into a counter-current of heated air. In the Just roller process, on the contrary, the layer of milk on the cylinder is rapidly giving off water vapour while being heated, and the atmosphere of steam so produced on its exposed surface acts as a protective against oxidation.

Supplee and Dow (1926) undertook to clear up the disputed point by biological experiments. They fed groups of guinea-pigs with two distinct basal rations, and found that the addition to either of as much as 80 c.c. of reconstituted spray process milk per diem was insufficient to protect the animals from scurvy. On the other hand, dry milk made by the Just roller process, and stored in air at room temperature for two years, still had an antiscorbutic potency roughly equivalent to that reported for fresh milk produced at the same time of the

year; frequently 60 c.c., and always 80 c.c., of this reconstituted product sufficed to protect the animals from scurvy.

Swiss dried milk, according to Hottinger (1927), contains its full quota of vitamin C.

By Other Processes—(a) Pasteurisation.—A good deal of destruction of vitamin C occurs during the pasteurising process, chiefly owing, as shown by the experiments of Kohman (1923), on a large milk plant at Washington, to the setting free of dissolved oxygen during the process. Van Leersum (1926) points out that saturation of the milk with air might be avoided by using vacuum milking-machines, by replacing surface coolers with submerged coils, and by a shortened time of transport, and in full cans so that shaking is prevented. Since the milk used in making ice-cream is pasteurised, ice-cream, according to A. H. Smith (1922), contains practically no vitamin C, apart from the question whether it is destroyed by freezing, which has not yet been decided.

Seligmann and co-workers (1926) however concluded, from several experiments in which guinea-pigs were fed with Berlin market milk which had been pasteurised in various ways, that pasteurisation has no noteworthy effect in decreasing the content of antiscorbutic vitamin, and their results are confirmed by those of Bakke (1930). Miura (1929) also states that commercially pasteurised milk contains an appreciable amount of vitamin C.

(b) Concentration.—According to Lavialle (1926), a sterilised non-sweetened concentrated milk, in the manufacture of which air is excluded as far as possible, preserves its antiscorbutic potency, and Bakke (1930) also states that sugared condensed milk supplies an amount of vitamin C sufficient to prevent scurvy.

(c) Electrical Treatment.—Van Leersum (1926) states that milk sterilised by exposure to a current of 2000 volts with copper electrodes is completely deprived of vitamin C, but with carbon electrodes, provided the milk used be fresh and contains but little oxygen, the vitamin destruction is much less.

(d) Destruction of Vitamin C by Canning.—Canned goods have been suspected of losing their vitamin C content on the grounds that they are often subjected to higher temperatures for longer periods than the home-cooked article. Spinach, for example, in No. 2 cans is usually cooked for 70 minutes at 240° F.

Kohman, Eddy, and Carlsson (1923, 1924) have worked out the vitamin destruction in various vegetables and fruits, including cabbage, spinach, and apples. It has been pointed out by Kohman (1923) that the oxidation factor in canning is difficult to exclude. Eddy and co-workers (1924) believe that the destruction of vitamin C in canning is largely an oxidative process, and that, if oxygen be excluded, heat has little destructive effect.

They found that canned cabbage, even when processed as long as 60 minutes at 212° F. in one case and 45 minutes at 240° F. in another, protected guinea-pigs in a dosage of 5 grms. and 4 grms. daily respectively.

That is to say, canned cabbage, cooked as long as open kettle home-cooked cabbage, and at the same or even higher temperature, was actually from 4 to 5 times as antiscorbutic as the home-cooked article, and from one-fourth to one-fifth the value of raw cabbage.

Eddy's results on cabbage were confirmed with spinach. Canned spinach proved protective in doses of 1 gram daily, whether processed 70 minutes at 240° F. or 120 minutes at 240° F. They showed further that the time of blanching was apparently more significant than the duration of the heating process after sealing—a fact which has been further confirmed by the study of canned apples. Eddy tested different methods of canning apples:

- (i) The ordinary commercial process—covering with hot water, exhausting, sealing, and processing for varying periods at 212° F.
- (ii) Immersing in 2 per cent. NaCl for several hours (brine treated).
- (iii) Washing out oxygen with nitrogen.

The results showed that the brine treatment was more effective in preserving the vitamin C than the nitrogen washing, and that apples treated in this way were richer in vitamin C than those treated without previous soaking in salt solution. Eddy believes that the brine treatment eliminates a destructive factor that is not molecular oxygen and that prevents discoloration.

The vitamin content of canned pear has been worked out by Craven and Kramer (1927); they find that the open-kettle method of home canning destroyed the vitamin C completely, while most of it is destroyed by the cold-pack method. Canned tomatoes have been found by Hess and Unger (1919) to preserve their activity for three years, and Kohman and co-workers (1926) state that they are equivalent to raw tomatoes.

Delf (1924) states that tomato purée, prepared by simmering tomatoes in their own juice at 80° to 90° C., straining the thickened juice, and sealing in cans (which involves further heating at 100° C. for 5 to 10 minutes), had been reduced in antiscorbutic value by 85 per cent.

(e) *Concentration*.—According to Goss (1925), commercially concentrated orange juice is a good source of vitamin C. The methods of Zilva (1922–1928) for the concentration of lemon juice have made available a powerful antiscorbutic remedy. Zilva has reported a case (1924) of scurvy in an infant of 8 months, which was cured remarkably rapidly by administration of large quantities of concentrated and decitrated lemon juice. The child was given an amount equivalent to about 15 lemons in the first 24 hours, and within 10 days received a total amount equivalent to about 48 lemons.

(f) *Preservative Agents*.—The difficulty in preserving the juices of fruits and vegetables lies in the fact that they are particularly susceptible to fermentation by moulds and bacteria.

Unfortunately, recent studies by Williams and Corran (1930) indicate that those substances which exert the strongest preservative effect against gross fermentation possess the greatest destructive action on the antiscorbutic vitamin.

Potassium metabisulphite was found by them (contrary to the results of Goss, 1925) to have a destructive action upon vitamin C at room temperature.

Sodium benzoate was also found destructive, though de Blasi (1926) stated that when added to the diet as a 1 per cent. solution it did not affect the vitamin C content of cabbage and clover.

Sodium bisulphite, in the proportion of 6 parts per 1000, is, according to Delf (1925), destructive to the antiscorbutic potency of lemon juice, the diminution of activity being 50 per cent. after 2 years, 80 per cent. after 4½ years. *Chlorine*, according to Liotta (1925), in the proportion of 5 per cent., preserves the antiscorbutic potency of orange and lemon juice fairly well. Benzoic acid is stated by Wulffing (1926) to fail to preserve the vitamin C of carrot juice.

Formic acid and oil of cloves were also found detrimental by Williams and Corran (1930), while lemon rind oil, though less destructive, did not satisfactorily prevent fermentation.

(g) *Effect of Acids in Destruction of Vitamin C*.—The optimal zone of stability of vitamin C in lemon juice is, according to Williams and Corran (1930), that of the natural acidity of lemon juice, i.e. at pH 1.6 to 2.2. Lemon juice adjusted with HCl to pH 1.6 preserves its antiscorbutic activity for 14 months at ordinary temperatures.

Zilva (1925) has shown that decitrated lemon juice in acid solutions (pH 2.2 to 2.4) loses less vitamin C on being boiled in the presence of air than in a solution of pH 6.8.

Acidulation of certain vegetable juices (e.g. the juice of potato) has been found to increase their antiscorbutic potency, and Bezssonoff (1922) attributes this effect to a destruction or "paralysis" by citric or tartaric acid of the action of oxidising diastases which would otherwise have a destructive effect upon the vitamin C present in the vegetable juices.

The same explanation (destruction of oxidising diastases) was offered by Delf and Skelton (1908) for the fact that cabbage, plunged for a few moments into boiling water, preserves its antiscorbutic power longer than cabbage exposed to the air without such immersion.

(h) *Alkalies*.—In the presence of air or oxygen, alkaline solutions have a destructive action on vitamin C, 80 per cent. of activity being lost in solutions of pH 12 in 30 minutes at room temperature.

(i) *Ultra-Violet Rays*.—It has been shown by various workers that the synthesis of vitamin C is quite unconnected with ultra-violet light. Eggleton and Harris (1925) found that oats germinated in the dark, and producing therefore a greater length of shoot than when exposed to light for the same length of time and under the same conditions, produced a correspondingly greater amount of vitamin C. Ultra-violet light had neither curative nor preventive action on the onset of scurvy, nor was the antiscorbutic potency of food exposed to ultra-violet light increased. Janet Clark (1925), Honeywell and Steenbock, Krizenecky (1926), and Milanesi (1928) reached similar conclusions.

Whether irradiation causes actual destruction of vitamin C has been the subject of difference of opinion. Hottinger (1927) states that milk which has been irradiated contains as much vitamin C as is present in the milk before irradiation.

Neither Spinka (1924) nor Zilva (1919) found any destruction of vitamin C in irradiated lemon juice.

Reyher and Miller (1926), and Reyher (1927), on the other hand, have shown that by irradiation of milk in open vessels complete destruction of all the vitamin C in the milk can be brought about. If irradiated through a quartz vessel, only slight loss of the vitamin C in the milk takes place. Huldchinsky suggests that this loss is due to the effect of heat from the effect of irradiation, but it has been shown by many observers that heat only acts in so far as it increases the rate of oxidation, and it only causes a partial loss of vitamin C in the quartz vessel, whereas there is a complete destruction in the open one.

(F) **COLOUR TESTS FOR VITAMIN C (not strictly specific)**—(1) **Bezssonoff's Reaction**.—Bezssonoff (1921, 1922) made the observation that a number of antiscorbutic substances gave a blue coloration with phosphomolybdotungstic acid in an acid medium (Folin's reagent for phenol), and he suggested the use of this reagent for the detection of the antiscorbutic factor.

This reaction has been criticised by Wedgewood and Ford (1924), by Kay and Zilva (1923), and by Connell and Zilva (1924) on the score that it is given by substances which have no antiscorbutic potency, and that it is not given by substances which have.

Bezssonoff (1924) has proposed a modification which, he claims, renders it more specific. By heating the liquid in a slightly acid medium he states that a more intense reaction is given if the antiscorbutic factor is present. If heating decreases the reaction he considers the test negative. Although this modification makes the test more sensitive, it is still not, according to Frank (1925), rigorously specific.

(2) **Zilva's Reducing Agent**.—Zilva (1925) has found the reduction of ammoniacal silver nitrate in the cold to coincide with the presence of the antiscorbutic factor. His later investigations on the two factors in vitamin C, and the discovery that their rates of destruction are different, have led him to conclude that the reducing properties of active solutions cannot be taken as criteria for their activity.

LX. VITAMIN C AND METABOLISM.

The disturbances of metabolism in vitamin C deficiency, so far as they have been investigated, appear to be slight, except, as emphasised by Mouriquand and Leulier (1927), as regards iron and cholesterol. Unlike vitamin B, vitamin C appears to be unconnected with

loss of appetite, and the effects of its deficiency bear no close relationship to those of inanition (Mouriquand and Michel, 1921).

The findings of various observers are not in complete agreement as to the exact function played by vitamin C in metabolism.

Toverud (1923) believed the symptoms of scurvy to arise primarily from a disturbance of calcium metabolism, since he found a low Ca retention, associated with a high Mg retention, but other workers, particularly Palladin and Sswaron (1924), have found practically no variation in the blood calcium in scurvy. The theory of Bezssonoff (1928) that the rôle of vitamin C is chiefly that of facilitating the excretion of toxic substances, such as paracresol and phenol, is interesting, but has received no definite support from other sources.

(A) **BASAL METABOLISM.**—According to Abderhalden (1921) the production of CO_2 by a scorbutic animal does not differ from the normal, nor is it altered by the addition of anti-scorbutic substances. According to Jarussowa (1926) the gaseous exchange is slightly diminished. Tissue respiration is stated by Randoin and Simonnet (1927) to have a tendency to diminish in manifest scurvy, but the tendency is not constant. These workers also state that lemon juice, even in high dilution, has an accelerating effect on tissue respiration, both in normal and scorbutic tissues, though more markedly in the latter.

(B) **NITROGEN METABOLISM.**—According to Caridroit (1924) and Schepilewskaya and Jarussowa (1925, 1926) there is a slight variation in the urinary nitrogen during the course of scurvy. They state that, after an initial decrease, the excretion of N increases *pari passu* with the loss of weight, as if the animal, having lost the power to utilise the protein in its diet, were drawing on its own protein reserves. No indications of such disturbance, have, however, been found by Shipp and Zilva (1928). They considered it unlikely, judging from their results, that the development of scurvy is associated with an increased C/N ratio in the urine.

The blood urea, according to Randoin and Michaux (1925), is raised from 0.36 to 0.37 to 0.742, concurrently with an increase of the urea content of the tissues already reported by Lewis and Karr (1916).

(C) **CARBOHYDRATE METABOLISM.**—According to Randoin and Michaux (1925) and Mouriquand and Leulier (1927) the metabolism of sugar is unaffected in scurvy, except for a diminution in the glycogenic reserve. Their findings are not in agreement with those of Palladin (1924) and Abraham (1928). The latter states that during the disease the rise in blood sugar after a meal of glucose is delayed, reaching a maximum in 2 hours, while, after the disappearance of scurvy, this maximum was reached in 1 hour.

(1) **Acidosis.**—Wright's description (1897) of scurvy as an "acid intoxication" has been disproved by many workers. The diminished alkalinity of the blood found by Birt (1907) was only slight, and could probably be explained, as suggested by MacRae (1902), by the associated anæmia. No acidosis was found by Funk (1916), McClendon and co-workers (1919), Mouriquand and Michel (1921), or Lepper and Zilva (1925).

It has recently been pointed out by Mouriquand, Leulier, and Sedallian (1927) that the symptoms of scurvy cannot be due to acidosis, as suggested by the earlier workers, since the fall in the alkaline reserve of the plasma did not begin until after the 27th day, *i.e.* until some time after the typical early symptoms of scurvy had developed. In their experiments determinations of the pH of the plasma of guinea-pigs indicated that in C avitaminosis no appreciable deviation from that of normal animals occurs (except immediately before death, when a fall to pH 7.2 was recorded).

(2) **Lactic Acid Metabolism.**—Rosenwald and Morinaka (1926) carried out experiments from which they concluded that the excretion of lactic acid was increased in dogs on vitamin-deficient diets. Shipp and Zilva (1928) have since pointed out that these experiments were

unsatisfactory, since the diet used was imperfect in a number of respects. They state that the lactic acid excreted is proportional to the volume of the urine, its concentration in the urine remaining practically constant at 0.01 per cent. Since the pre-mortal phase of scurvy is usually associated with polyuria, the actual excretion of lactic acid is increased, but Shipp and Zilva have shown that a similar increase in the volume of the urine takes place in guinea-pigs declining in weight on a restricted diet containing a sufficiency of the antiscorbutic factor. It is therefore not specific to scurvy in guinea-pigs.

(D) **CHOLESTEROL METABOLISM.**—According to Mouriquand and Leulier (1927) the disturbance of cholesterol metabolism is manifested chiefly in the adrenals. They found the cholesterol content of the blood to be a very variable figure in normal animals, and in scorbutic animals no change could be found in the average of a series of analyses. However, on examining the organs, it was observed that whilst the cholesterol content of the liver, lung, and spleen remained normal in scorbutic animals, that of the suprarenal gland fell by as much as a half, three-quarters, or even more, and if in addition the animals were tuberculous, this fall was still more severe. Simple inanition for 3 to 4 days caused a hypercholesterolaemia, with no effect on the cholesterol content of the organs.

In the kidneys, according to Randoin and Michaux (1928), the cholesterol content remains practically unchanged. The latter workers also state that the cholesterol content of the liver of scorbutic guinea-pigs seems, on the average, to be higher than for normal pigs, on both the fresh and dry basis, but individual variation makes it impossible to draw definite conclusions. The fatty acids definitely increase with advance of scurvy, however, so that the lipocytic coefficient cholesterol fatty acids $\times 100$ declines.

In the spleen also they find that in the most advanced stage of the disease the cholesterol and fatty acid content drop to a low level (both fresh and dry basis), and there is a decline in the lipocytic coefficient.

(E) **EXCRETORY FUNCTION OF THE LIVER.**—According to Saiti (1929), who has tested the excretory function of the liver in vitamin C deficiency by injecting organic dyes into the blood stream, and determining the amount of dye in the bile after certain time-periods, a slight disturbance occurs, differing in character from that which takes place in vitamin A and B deficiency.

Guinea-pigs were used for these experiments, and the dye employed was cocchine 2 B. A much greater percentage of the total amount of dye injected was excreted in the first 2 hours in the deficiency animals than in the controls.

(F) **MINERAL METABOLISM** — (1) **Iron.**—Mouriquand and Leulier (1927) regard the disturbance of iron metabolism in scurvy as important and characteristic. They found that there was a decrease in the iron content of the blood, running parallel with the degree of anaemia, and the extent of the clinical signs. The amount of iron fell to nearly half the normal value and rapidly returned (5 to 15 days) to normal when lemon juice was added to the diet.

Randoin and Michaux (1927), however, regard the liver as the organ in which the diminution of iron is most marked. They observed no change in the percentage of iron in the spleen; only a very slight diminution, if any change, in the blood; and, after the twentieth day on the vitamin C-deficient diet, a definite diminution in the iron content of the liver, which finally reaches an average of 0.45 mgrm. per 100 grms. as compared with the normal average of 2 mgrms. per 100 grms. of tissue. They conclude that the important reserve of organic iron in the liver tends to become exhausted when the diet is totally deficient in vitamin C.

(2) **Calcium.**—The results of earlier workers on the metabolism of Ca in scurvy were summarised by Hess (1920) in the words, "The only point in which they agree is that during the manifest stage the Ca balance is positive."

According to Palladin and Sswaron (1924) the blood calcium varies very little, though there is a decreased excretion.

Bauman and Howard (1917), on the other hand, state that the elimination of calcium is increased.

Popowa (1928) has pointed out a connection between the Ca balance and the occurrence of tuberculosis in these animals. He showed that the blood Ca is influenced by neither the absence nor presence of vitamin C, but rather by the nutritional disturbance produced by the restricted diet. When normal guinea-pigs receive an abundance of vitamin C and are infected with a virulent culture of tubercle bacilli, the disease runs a relatively benign course and the blood Ca is normal or increased. If, however, the tuberculous infection occurs in scorbutic guinea-pigs during the period of Ca demineralisation, the disease runs a virulent course to a rapidly fatal termination.

Popowa suggests, therefore, that the Ca balance, plus the weight and temperature curves, may therefore be utilised as a valuable aid in the prognosis of tuberculosis.

(3) **Phosphorus.**—Palladin and Bauman both state that the excretion of phosphorus is diminished.

(4) **Sulphur and Potassium.**—According to Bauman and Howard there is a decreased elimination of these elements.

(5) **Magnesium.**—Increased elimination of magnesium is reported by Bauman and Howard. It should be mentioned that Howard and Ingvaldsen (1917), working with monkeys, have failed to confirm the above modifications of mineral metabolism.

(G) **STORAGE OF VITAMIN C.**—The storage capacity of the body for vitamin C is not great. The onset of scurvy is not delayed in guinea-pigs which have been given an abundance of lemon juice before being placed on a scorbutic diet.

It will be seen, however (p. 334), that the tissues of animals which have a very low requirement for vitamin C have some antiscorbutic potency even when they are placed on a diet which would be scurvy-producing in a susceptible animal.

The explanation of this fact is not entirely clear. Randoin and Simonnet suggest that these animals may be able to synthesise vitamin C from substances possessing no antiscorbutic potency.

(H) **ABSORPTION OF VITAMIN C.**—Vitamin C can be absorbed intraperitoneally (Holst and Frölich (1912), Lesné, Christou, and Vagliano (1923), Lesné and Vagliano (1923)), or intravenously (Hess and Unger (1918)).

Subcutaneous administration was stated by Harden and Zilva (1919) to be unsuccessful, though Lesné and co-workers found that it absorbed by this method. Absorption through the skin in sufficient quantity to protect a guinea-pig from scurvy is reported by Richter (1927).

Young guinea-pigs fed on a scorbutic diet had an area of skin on their back shaved, and over this area was strapped a compress which was soaked 4 times a day with the juice expressed from spinach.

Richter states that this treatment kept quite a large proportion of the animals alive, although controls receiving the same diet invariably died within a month. The addition to the juice of a salt mixture produced even better results. If the application of the remedy was delayed for not more than 6 days after the beginning of the scorbutic diet, symptoms of scurvy did not appear.

[The details of the experiment given do not appear to exclude the possibility of the animals getting some of the juice into their mouths. Though the compress was mostly covered over with strapping, a window was left in the middle through which the juice was introduced, and this might have been within reach of their paws.]

LXI. PHYSIOLOGICAL ASPECTS OF VITAMIN C DEFICIENCY.

(A) **SCURVY**—(1) **Etiology**.—The origin of scurvy has been less disputed than that of the other recognised deficiency diseases, but various theories have been advanced at different times.

(a) **FOOD DEFICIENCY**.—The connection between food and the disease now known as scurvy (probably the “stomacace” of Pliny) seems to have been realised almost from its first recognition. Pliny states that the disease existing in the army of Germanicus was cured by eating a certain vegetable.

The necessary food restrictions imposed upon pioneers of navigation further emphasised the connection, for the sailors of Bartholomew Dias (1486), Vasco da Gama (1496), Raymond (1591), and Lancaster (1691) suffered to a considerable extent from lack of fresh provisions and, consequently, from scurvy.

Van Andel (1927) states that scurvy was clearly recognised in the fifteenth century, and that even laymen (especially travellers and sailors) were acquainted with scurvy as a dietary disease, prevented or cured by various fresh fruits and vegetables.

The later navigators, Cartier, Dampier, Anson, and Captain Cook, proved the efficacy of fruit juices, vegetables, and fresh meat. Captain Cook (1772) emphasised the value of supplies of sweet-wort, sour-kroot, and fresh animal and vegetable food, and for his successful discovery of a method of preventing scurvy was awarded the Copley Medal of the Royal Society. Venette (1671) stated that oranges and lemons contained something which was directly opposed to the causes of scurvy.

The writings of Lind (1797), with their clear and scientific record of scurvy, led to the better feeding of sailors and the gradual disappearance of scurvy from the naval services. In spite of these early indications the etiology of scurvy as a deficiency disease did not remain undisputed, and in the early part of the nineteenth century the time-honoured view was no longer universally held.

(b) **DEFICIENCY OF MINERAL SALTS**.—A deficiency of potassium salts in the food was believed by Garrod (quoted by Jackson and Harley, 1900) to be the cause of scurvy.

Calcium deficiency was advanced as the active agent by Pitz (1918), but he was unable to bring forward convincing evidence, and it had already been shown by Holst and Frölich (1912) that treatment of scurvy with calcium salts was ineffective.

(c) **ACIDOSIS**.—A diminished alkalinity of the blood, due to the absence of vegetable acids, such as malates, citrates, and lactates in the food, was suggested by Ralfe (quoted by Jackson and Harley, 1900). Jackson and Harley state that it was proved by analysis that the alkalinity of the blood was not diminished, and that the theory had therefore to be abandoned.

Sir A. E. Wright (1897) described scurvy as an “acid intoxication which eventuates in a defect of blood coagulability.”

Examination of the alkalinity of the blood by Macrae (1908) and Birt (1907) showed very little diminution, while the presence of ante-mortem thrombi in the heart and great blood vessels pointed to an increase rather than a decrease of coagulability. Stockman, moreover, has stated that the blood in scurvy coagulates normally.

The acidosis theory was revived, under the name of “acidosis intoxication,” by Morgan and Beger (1915), but their evidence was contradicted by Mouriquand and Michel (1921), who found that the administration of alkali had no effect on the course of the disease.

According to Mouriquand, Leulier, and Sedallian (1927), however, the alkaline reserve is diminished in scurvy, as in inanition, but remains normal if sodium bicarbonate is added to the ration.

(d) TAINTED FOOD.—The view that scurvy is essentially due to poisoning by ptomaines of tainted animal food was first put forward by Torup of Christiania (quoted by Jackson and Harley, 1900), and his view was upheld by Jackson and Harley themselves. In support of their theory they quoted the Nares polar expedition, 1875, when in spite of a daily ration of lime juice scurvy developed with great severity. It was pointed out by Chick, Hume, Skelton, and Smith in 1918, however, that the failure of lime juice on this occasion was due to the fact that the antiscorbutic value of fresh limes is very low—about that of lemon juice.

Further evidence was adduced by Jackson and Harley to the effect that the crews of the *Eira*, with Neale (1896) as its medical officer, lived for nine months “under conditions of considerable privation and hardship,” and with no lime juice, and yet developed no scurvy. Jackson and Harley’s statement that these men “lived on freshly-killed bear meat and walrus meat” is its own explanation of the source of the antiscorbutic principle. That this explanation was beginning to be realised by 1906 is shown by the conclusion reached by Macvicar from an examination of the etiology of 47 cases of scurvy in Cape Colony: “Food may be in perfectly good preservation and yet lack the antiscorbutic power of ‘fresh’ food. Thus men on polar expeditions using tinned vegetables and even lime juice became scorbutic, but lost their symptoms of scurvy as soon as they had access to ‘fresh’ seal meat.”

(e) BACTERIAL INFECTION.—A certain number of workers have regarded scurvy as a disease of bacterial infection.

In 1909 Okada and Saito arrived at the conclusion that a micrococcus could be isolated from the blood of scorbutic patients and was the actual cause of the disease.

Coplands (1904) and Jackson and Moody (1916) have also upheld the bacterial theory, and have brought forward some experimental evidence in support of it.

Jackson and Moody noted small stained bodies, which they suspected were bacteria, in the lesions of guinea-pigs suffering from scurvy. They made cultures on blood agar and shake cultures in ascites dextrose from a material obtained by grinding up the diseased joints, muscles, and lymph glands of scorbutic guinea-pigs. After 20 hours’ incubation at 37° C. small greenish colonies were present on the plates and small pin-point colonies in the shake cultures. Their conclusions were as follow :

“Cultures of crushed scurvy tissue resulted in the isolation of a diplococcus of low virulence with a tendency to form chains and to produce a green coloration on blood agar. Pure strains of these organisms inoculated into the circulation of guinea-pigs and rabbits living under ordinary conditions (a mixed diet, consisting of green vegetables, hay, and oats) gave rise, in most instances, to hæmorrhagic and other lesions in the bones, joints, vessels, lymph glands, or gums. Streptococci of the same type as those injected were recovered from the lesions in these animals as late as 40 days after a single intracardiac or intravenous injection. Bacteria resembling those described were frequently seen in the microscopical sections of the scurvy lesions. In animals whose resistance was kept high by proper feeding, the lesions produced by injection of these streptococci did not progress so rapidly as in those on an unbalanced diet.

“Cultures of the heart blood of the affected guinea-pigs were, however, sterile, and passage of blood from an affected to a normal animal failed to reproduce the disease.”

An explanation of their results is suggested in the Medical Research Council Report (1924–1928) that any animal in a scorbutic condition due to dietary deficiency will be a ready prey to secondary infection.

(f) INTESTINAL STASIS.—McCollum and Pitz (1917) suggested that scurvy in guinea-pigs was due to chronic stasis at the level of the cæcum, brought on by an unsuitable diet. They found that a diet of milk and grain, while adequate for the rat, frequently resulted in scurvy in the guinea-pig. They concluded, therefore, that the inadequacy for the guinea-pig must be

due to lack of "roughage." McCollum (1918) believed that he could cure scurvy by purgatives and laxatives, Pitz (1918) with lactose.

These views were criticised by numerous workers, including Cohen and Mendel (1918); Chick, Hume, and Skelton (1918); Hess and Unger (1918); Torrey and Hess (1918); Harden and Zilva (1918); Hess (1916); and later knowledge of the low antiscorbutic potency of milk, and the different requirement of the rat and the guinea-pig for the antiscorbutic factor, have finally disposed of the theory of intestinal stasis as the causative factor in scurvy.

(g) VITAMIN DEFICIENCY.—The inclusion of scurvy in the list of diseases due to a deficiency of vitamins dates from the investigations of Holst and Frölich (1907, 1912) into experimental scurvy in guinea-pigs. They recognised that a disease which developed in guinea-pigs fed on cereals and water, and which had been already described by Smith (1895), showed a marked resemblance to the scurvy of sailors. They showed that by supplementing the grain diet by fresh fruits, vegetables, and fruit juices, scurvy could be prevented and cured. They also noted that the "antiscorbutic substance" in which these articles of food were rich was soluble in water and alcohol, and was very unstable to heat, but more stable in the presence of organic acids such as those which are present in oranges and lemons. Finally they stated that these acids possessed no antiscorbutic potency in themselves, and that they were not even necessary to the action of foods possessing such potency.

Funk (1914) had no hesitation in classing the antiscorbutic substance with the group of "vitamines," and the experimental work on guinea-pigs and monkeys carried out by Chick and co-workers (1918), Stefansson (1918), and others confirmed his view. In 1918 the Royal Society Food (War) Committee issued the following data concerning the causes of prevention of scurvy:

"Scurvy, like beri-beri, is a 'deficiency disease,' and is due to the long-continued consumption of food lacking in an accessory food substance or vitamin. The view that scurvy is due to tainted food must be abandoned. This vitamin is contained in a number of fresh foods; in largest amount in oranges, lemons, and fresh green vegetables; in considerable amount in roots and tubers, such as swedes, potatoes, etc.; and in small quantities in fresh meat and milk. It is deficient in all dried and preserved foods. It is destroyed by prolonged heating, such as takes place during stewing. Thus, potatoes in stews would be devoid of vitamin, but if boiled rapidly will still contain some quantity. Alkalies rapidly destroy antiscorbutic properties. Therefore soda should not be added to the water in which vegetables are soaked or boiled."

(2) **Clinical Aspects of Scurvy.**—(a) TYPICAL SCURVY in adult human beings is rarely found nowadays, since the only members of the community likely to be completely deprived of the antiscorbutic factor are aware of the methods of preventing such deprivation.

The chief features of a case of well-developed scurvy are, however:

- (1) Gingivitis—a spongy condition of the gums, with loosening of the teeth, which may go on to ulceration.
- (2) Hæmorrhagic and painful swellings near the joints, especially the knee joints.
- (3) Petechial ecchymoses of the skin.
- (4) Anæmia.

(b) PRESCORBUTIC CONDITIONS.—A partial deficiency of vitamin C extending over a long period may produce a condition which, though difficult to diagnose as scurvy, has been called by Mouriquand a "pre-deficiency" state. In animals, according to Mouriquand (1930), no active symptoms of scurvy are recognisable when their diet is such as to induce scurvy only after a long latent period, but such symptoms can be easily precipitated, for instance, by feeding the animals on thyroid.

Weill and Mouriquand (1919, 1922) have described these conditions in soldiers whose diet

was deficient in fresh vegetables. They are characterised by debility, with slight pains in the lower limbs, bleeding from the gums, and intestinal disturbances.

A "prescorbutic myopathy" in children, which may simulate subacute rheumatism, has also been described by Friderichsen (1928). In all his cases there eventually appeared some evidence, clear or equivocal, of a scorbutic condition.

Hahn (1930) states that pains in the shins and bleeding gums are frequently seen in children's clinics in Hamburg, from March to May, when the supply of antiscorbutic foods is at its lowest. That such a prescorbutic condition may be precipitated into typical scurvy by the onset of some infectious disease is suggested by Woringer and Sala (1928). They point out that in the epidemics of scurvy observed in certain countries during and after the war, an infectious disease such as influenza, dysentery, or diphtheria was often followed by the appearance of the osteo-hæmorrhagic syndrome, and that in children in the prescorbutic stage, vaccination against smallpox may give rise to subperiosteal and other hæmorrhages. In their own cases 4 infants developed infantile scurvy about 6 weeks after the onset of whooping-cough. The symptoms disappeared on the administration of fruit juice, though perhaps more slowly than they would have done in uncomplicated scurvy. The diet of these children was the same as that of the others in the clinic among whom there were no cases of scurvy, so that although the milk, in consequence of repeated pasteurisation, might have lost some of its vitamins, it was probable that the infection of whooping-cough played a part in the development of the scurvy.

(c) **LATENT SCURVY.**—Although fully developed scurvy is a rare disease, there are indications that it may occur more frequently than has been supposed, in a form so mild as to escape recognition. Meulengracht (1927) has reported 8 cases in Denmark, which he believes to represent scurvy due to unemployment and poor housing conditions. The patients were all men who were either unmarried or living apart from their wives, and inhabiting a single room, in which, as a rule, there were no cooking facilities. None of them ever partook of potatoes, fresh vegetables, or fruit. The early symptoms were fatigue, drowsiness, depression, irritability, palpitations, and loss of appetite. Later they developed the characteristic hæmorrhages and swollen gums.

Twenty-two similar cases, all but one of which were considered to be due to a vitamin C deficiency brought about by the modification of the diet for intestinal disease, have been reported by Ohnell (1928). The symptoms were definite, but the predominant feature was a gingivitis, localised to the proximity of the teeth and accompanied by intensively swollen interdental papillæ, though rarely by marked bleeding. Cutaneous hæmorrhages, almost exclusively confined to the lower extremities, were noted in most cases; these were usually isolated, but in a few cases were confluent. Hæmorrhages in other organs were rarely observed, hæmaturia occurring only in one and slight hæmoptysis in two patients. Tests for occult melæna were always negative. In two cases there was marked œdema of the lower limbs. Rheumatic pains and slight anæmia were often present, and nervous disturbances were very frequent. The so-called Hess test (cutaneous hæmorrhages appearing in the fold of the elbow and perhaps the forearm and hand when the venous circulation to the arm is obstructed) was utilised. Ohnell adds that the presence of gingivitis and cutaneous hæmorrhages, combined with a history of vitamin C deficiency, are the chief factors in diagnosis, which may be aided by a radiological examination of the teeth for the typical scorbutic changes which are often seen.

Gichner and Sherry (1930) also describe cases of scurvy due to erroneous nutritional habits.

(d) **INFANTILE SCURVY.**—The bony lesions characteristic of scurvy in infants have in the past led to some confusion between scurvy and rickets. Moeller, for instance, in 1856 gave a clear description of infantile scurvy, but called it "acute rickets."

Barlow (1894) showed the relationship between infantile scurvy and the scurvy of adults. He stated his opinion that "the boiling of cows' milk and prolonged sterilisation (especially at high temperatures) lessens in some degree its antiscorbutic quality." The practice of pasteurising milk for infant feeding was also held responsible for many cases of infantile scurvy by Neumann (1902) and Heubner (1903). An outbreak of mild and subacute infantile scurvy among infants who had been fed for several months upon cows' milk previously heated to 63° C. for 30 minutes was found by Hess and Fish (1914) to disappear when orange juice was restored to the diet or when raw milk was substituted for the pasteurised.

A clinical picture of infantile scurvy was given by Colman (1903): "A fairly-nourished child with a pale face, or, in the later stages, a peculiar earthy complexion, lies quiet without obvious discomfort as long as he is not touched, but directly any one touches him, or even approaches the cot, he begins to scream with fear or with pain. A characteristic attitude is seen as the child lies in bed; the legs lie flaccid, with the thighs abducted and the knees a little flexed. The bones of the limbs are tender, and usually there are local swellings due to subperiosteal hæmorrhages, most frequently just above or below the knee. Hæmorrhage into the orbit occasionally gives rise to proptosis and ecchymoses of the eyelids. If teeth have been cut, there are nearly always changes in the gums, swelling, and hyperæmia, or actual hæmorrhage. Epistaxis and hæmaturia are not uncommon; subcutaneous hæmorrhages, so common in adult scurvy, are only occasional in the infantile form."

According to Miyake (1923) the incubation period appears to be from 6 days to 5 months.

Hess (1917) describes two varieties of infantile scurvy which he calls "subacute" and "latent." The "subacute" form, which is frequently produced by an exclusive diet of pasteurised milk, is less obvious and less easily recognised than the classic florid type. It is distinguished by a group of incompletely developed symptoms which quickly disappear when orange juice or other specific therapy is given. The "latent" form is a state of malnutrition caused by a "negative balance of vitamins." It is rarely definitely diagnosed except by the sharp recession of symptoms following the administration of an antiscorbutic.

Hess's Cardio-Respiratory Syndrome.—Hess (1917) states that infantile scurvy is associated with cardio-respiratory disturbances, characterised by very rapid pulse and respirations. This cardio-respiratory syndrome is due to an involvement of the nerves, which, in conjunction with the changes in the optic disk and increased knee reflexes, prove that the nervous system is greatly involved. Radiograms show that the heart is frequently enlarged with a broadening of the shadow at the base.

(3) **Pathological Changes in Human Scurvy.**—These have not been investigated with the same detailed attention which experimental scurvy has received.

(a) **IN THE BONES.**—Swelling of the costo-chondral articulations, congestion and hæmorrhage of the bone marrow, and fragility and thinning of the bones are the chief changes found. In children, separation of the epiphysis is common, and the thinning of the bone leads to spontaneous fractures.

(i) *Radiological Appearances of Bones.*—A feature of the bony changes, as seen by X-ray examination, which distinguishes scurvy combined with rickets from pure scurvy has been described by Wimberger (1925). This sign consists of a dense edge or ring about ossification centres of the epiphyses when combined with the characteristic ground glass atrophy of their centres. This ring remains intact in combined scurvy and rickets, while in isolated rickets the epiphysis has a somewhat moth-eaten appearance with no ring shadow.

The so-called "white line" of Fraenkel is also used in the radiological diagnosis of scurvy. This is the presence of a dense transverse shadow at the epiphyseal margin of the diaphysis. Schwartz (1927) regards as equally important narrow bands of diminished density of bone structure immediately subjacent to the diaphysis of long bones, and changes in the epiphyses,

consisting of a rarefaction of the centre and consolidation of the periphery. He states that in radiograms taken many years after an attack of infantile scurvy the epiphyses may still preserve the appearance of rings of dense bone surrounding a central area of rarefaction.

Similar bony changes, as revealed by radiographic examination, are regarded as pathognomonic of the larval forms of infantile scurvy by Acuna and Casaubon (1929). These are (1) a dark wavy line, irregular in depth, at the junction of cartilage and bone; (2) a diminution of density at the junction of the diaphysis and epiphysis; (3) a ring or halo round the centre of ossification.

(ii) *Healing of Bone Lesions*.—According to McLean and McIntosh (1928) the most prominent change during the first week of treatment is the increase in depth and density of the zone of preparatory calcification, which may go on for 5 to 7 weeks. Calcification of elevated periosteum (from hæmorrhage) may be present after long periods of treatment.

Bromer (1928) states that transverse striations can be regarded as a measure of the growth of the bone after the onset of the disease.

(b) *CHANGES IN THE BLOOD*.—Anæmia is recognised as a more or less constant condition in scurvy, especially in chronic cases. Its exact cause has been variously interpreted: some observers, such as Hansmann (1922) and Keefer and Yang (1929), attributing it chiefly to undernourishment and intercurrent infection; others, like Höjer (1924), to insufficient red cell production; while Methner and co-workers (1930) believe that the bone marrow functions inadequately in the absence of vitamin C.

(i) *The Blood Vessels*.—Bierich (1919) has suggested that the essential lesion in human scurvy, as in guinea-pig scurvy (see Findlay, 1921), is an interference with the nutrition of the capillary endothelium.

Fatty degeneration of the capillary walls was observed by Hayem (1871) in examining cases of human scurvy during the siege of Paris, and similar changes were noted by Lasegue and Legroux (1871). These changes have been considered by other workers attributable to post-mortem decomposition, but similar appearances having been demonstrated by Findlay (1921) in experimental scurvy immediately after the death of the animal make this explanation improbable.

(ii) *Red Corpuscles*.—The human subject of scurvy suffers from a greater degree of anæmia than the experimental animal.

Philip Wales, in Ashurst's *International Encyclopædia of Surgery* (quoted by Jackson and Harley, 1900), drew attention to the reduction in the number of red corpuscles, and the majority of observers, including Wassermann (1918) and Shattuck (1928), have described a secondary anæmia with a relative lymphocytosis. Wassermann, however, found the conditions variable, the red cells being sometimes subnormal, under 2,000,000 per c.mm., and at others, especially during convalescence, raised to 7,000,000 per c.mm.

Hess and Fish (1914) and Brandt (1919) have reported that there is very little decrease in the number of red corpuscles in human scurvy.

According to Methner and co-workers (1930) the hæmoglobin is reduced so that a colour index of about 1 is the rule; abnormal variation in size of the red corpuscles is more pronounced than variation in shade, about 1 per cent. of the cells are polychromatophilic; and nucleated erythrocytes may appear in the peripheral blood.

Globular Resistance.—According to Randoin and Michaux (1929) there are wide variations in the resistance of the red cells.

Abraham (1928) states that the osmotic resistance of the red blood corpuscles was sub-normal in a case of sporadic scurvy which he investigated.

(iii) *Blood Platelets*.—According to Shattuck (1928) the blood platelets show no reduction in scurvy, while Methner states that they may show a moderate increase.

(iv) *White Corpuscles*.—Laboulbene (1900) found an increase in white corpuscles in human scurvy, and Wassermann (1918) observed sometimes a relative, sometimes an absolute, increase in the number of lymphocytes, with an increase of eosinophil cells. Methers also found a slight lymphocytosis with about 3 per cent. atypical lymphoid cells.

(v) *Blood Coagulation*.—The investigation of the earlier workers (Stockman, MacRae, Birt) already referred to led them to conclude that there was no diminution of the blood-coagulation time in scurvy, and Shattuck (1928) has come to the same conclusion.

The bleeding time as recorded in 8 cases, and the coagulation time as recorded in 12 cases, were within normal limits. Retraction of the clot was noted only twice. It was normal.

(c) *THE BONE MARROW*.—According to Vedder (1929) and Methers and co-workers (1930) the bone marrow in adult scurvy shows alterations characteristic of secondary anaemia, *i.e.* disappearance of fat and cellular hyperplasia. Nægeli (1923) and MacCallum (1928), on the other hand, have described a loss of blood-forming elements and an oedematous fibrosis.

(B) **EXPERIMENTAL SCURVY**.—The study of experimental scurvy dates from the classical publication of Holst and Frölich in 1912, showing that a condition of scurvy, similar to that of the human being, could be produced in guinea-pigs if they were fed solely on a diet of oats and bran. The disease becomes manifest after about 15 to 18 days. The chief symptoms are tenderness and swelling of the joints, evidenced by the "scurvy position" of the animal, in which it rests on its side, with the painful member held in the air. When the gums and jaw become painful, the animal lies down with the side of its face on the floor of the cage. Later the teeth become loose, solid food is refused, and if the scorbutic diet is continued the animals decline in weight and succumb with the manifestation of the characteristic post-mortem changes, namely, intramuscular and subcutaneous hæmorrhages, osteoporosis, displaced costochondral junctions, etc. If, on the other hand, the scorbutic diet is supplemented with an antiscorbutic food or preparation, the onset of the disease is prevented, or if the antiscorbutic substance is administered before the disease is too far advanced, it is mostly arrested and cured.

(1) **Requirements of Animals for Vitamin C**.—(a) *THE GUINEA-PIG* is particularly susceptible to deficiency of the antiscorbutic factor. Zilva (1925) states that, utilising the guinea-pig as a test animal, quantitative results can be obtained with an error of about 20 to 25 per cent.

According to Mouriquand and Bernheim (1925) the young guinea-pig is very sensitive, and also there are marked individual variations in different guinea-pigs.

(b) *THE MONKEY* is also susceptible, the time taken to develop the disease being about 2 months.

Zilva states that the advantage of utilising the monkey in experimental scurvy is that it is more suitable for curative work. The administration of 1 or 2 doses of a potent substance will make the disease clear up with astonishing rapidity. More dilute solutions may also be employed owing to the larger capacity of the monkey's stomach. On the other hand, the longer the time taken in inducing the disease, the greater variation in the individual disposition to it, and the higher cost of the animal and of its upkeep makes the monkey less suitable for experimental work than the guinea-pig, especially for quantitative work.

(c) *THE RAT* is remarkably non-susceptible to vitamin C deficiency, and has been found unsuitable for biological testing. It has been shown by Harden and Zilva (1918) and by Drummond (1919) that, though the requirement of the rat for vitamin C is less than that of man, monkey, or guinea-pig, it is nevertheless necessary for its normal development. It has been suggested by Parsons and Hutton (1924), Lepkovsky and Nelson (1924), and Parsons and Reynolds (1924) that the capacity of the rat for living without vitamin C may be due to the fact that the rat can synthesise it, for the liver contains vitamin C in abundance. This

faculty is not apparently possessed by the guinea-pig, since the liver of the scorbutic guinea-pig has no antiscorbutic potency, while that of the rat fed on a scorbutic diet has.

This theory receives some support from the investigations of Thurston, Palmer, and Eckles (1929) on the requirements of calves for vitamin C. They were able to demonstrate vitamin C in the livers of calves fed for a year on a diet capable of producing scurvy in guinea-pigs within 30 days. They found also that heifers fed from birth on a scorbutic diet secrete appreciable quantities of vitamin C in their milk, and that reproduction in cattle can apparently proceed normally in the absence of vitamin C.

(d) **THE PIGEON AND CHICKEN**, according to many workers, including Hoet (1923); Randoin (1923); Simonnet (1921); Sugiura and Benedict (1923); and Plummer, Rosedale, and Raymond (1923), have no very definite need for the antiscorbutic factor. The liver of the pigeon and chicken fed on a scorbutic diet have antiscorbutic potency, according to Carrick and Hauge (1925) and Hart, Steenbock, Lepkowsky, and Halpin (1924).

(e) **THE RABBIT**.—The young rabbit, according to Findlay (1921), Holst and Frölich (1907, 1912), and Randoin and Lomba (1923), is susceptible to scurvy, but the adult rabbit can reproduce and rear its young on a diet deficient in vitamin C. This resistance is attributed by Portier and Randoin (1920) and Portier and Lomba (1921) to coprophagy and the production of vitamins in the intestinal canal.

(f) **THE PRAIRIE DOG** (*Cynomys Ludovicianus*) is susceptible to about the same degree as the rat. McCollum and Parsons (1920) report that a young prairie dog increased in weight from 169 grms. to 690 grms. in 6 months on a diet which produced scurvy in guinea-pigs in 2 to 3 weeks.

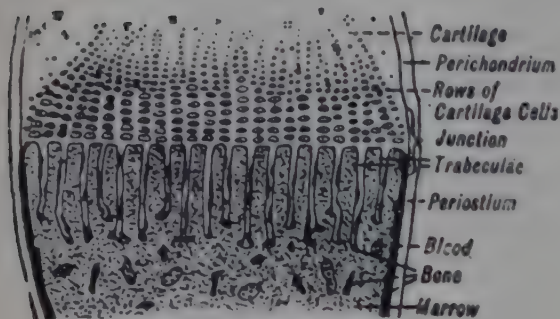
(2) **Pathological Changes in Experimental Scurvy**—(a) **IN THE BONES**.—The fragility of the bones which leads so often to fracture in cases of scurvy is apparently due to actual thinning produced by degenerative changes. The earlier workers, including Ingier (1913); Aschoff and Koch (1919); Hess (1920); Herzog (1921), etc., thought that the change in the primary calcification area was essentially a deposit of fibrin, but Höjer (1924) showed that the deposit consisted actually of inferior bone, due to the characteristic degeneration of osteoblasts. The extreme fragility of the bones might give the idea that they are decalcified, but, according to Mouriquand and Leulier (1928), X-ray photographs and chemical analyses show no evidence of deficiency of either phosphorus or calcium. The same conclusion was reached by Brouwer (1927), who noted a surprising uniformity in the mineral composition of normal and scorbutic tibias in guinea-pigs.

Early histological changes in the rib junctions have been described by Tozer (1918, 1921), as follows:

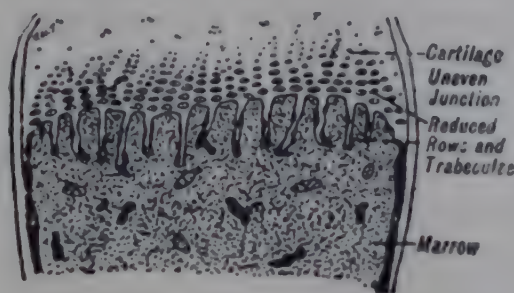
“The most noticeable departures from the normal are a variable amount of irregularity of the junction as a whole, slight disarrangement, and usually shortening of the rows of cartilage cells, and of the trabeculae; an increased amount of blood in the marrow cavity, and a reduction in thickness of the bone, especially near the junction.”

Later, these changes become more pronounced. The rib junction as a whole is disorganised, the rows of cartilage cells have lost their alignment and are scattered, and the trabeculae are shortened. The bone, which has become thin, is fractured on one or both sides of the junction, and, as a rule, quite close to it, the appearances suggesting that the cartilage is telescoped into the marrow cavity. There is hæmorrhage usually in the neighbourhood of the fracture into the marrow cavity and into the surrounding muscles. There is also a mass of fibrous tissue within the marrow cavity close to the junction and the fracture. The amount of this connective tissue varies considerably, from a few threads between the rather disorganised and possibly fractured trabeculae to a large plug filling the marrow cavity and displacing the marrow from its contact with the junction for a considerable distance. The marrow is not atrophied.

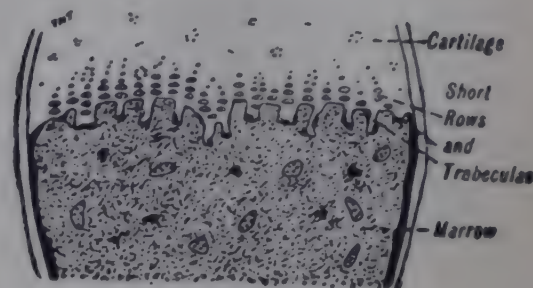
Degenerative changes were also observed by Meyer and McCormick (1928) in cartilage and bone. These workers observed proliferative changes in the costal cartilages, which produced an increase of calibre at the region of the costochondral junctions and an invasion of connective tissue into areas of degeneration. Changes in the osteoblasts and chondroblasts,



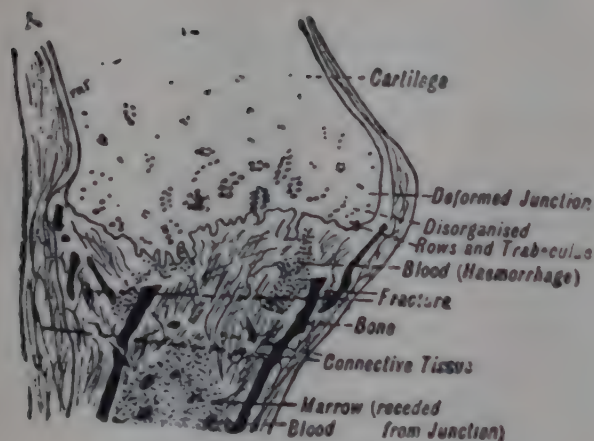
i. Normal Rib Junction.



ii. Nearly Normal Rib Junction.
"Incipient Scurvy."



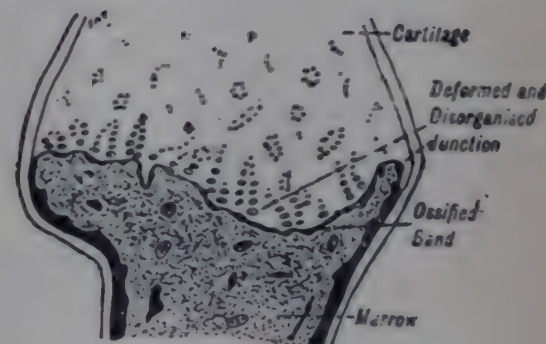
iii. "Definite Scurvy."



iv. "Acute Scurvy."



v. "Chronic Scurvy" (definite).
From *Med. Council Res. Report* (1928).



vi. "Chronic Scurvy" (acute).

in the form of irregularity of arrangement and diminution in size and number were described by Holst and Frölich (1907). It has been pointed out by Ingier (1913) that these changes occur quite independently of hæmorrhagic foci in the body, and Findlay (1921) suggests that these growing cells, concerned with the formation and repair of intercellular substance, are particularly susceptible to lack of vitamin C.

(b) THE HEART.—The earlier workers, including Leven (1871), Hayem (1871), Koch (1889), had reported varying degrees of fatty degeneration, accompanied by atrophy. Hyperæmia and atrophy, together with increase of connective tissue, were observed by Sato and Nambu (1905), while Hart (1909) and Hart and Lessing (1913) found some calcification of heart muscle.

The changes in the heart muscle of guinea-pigs dying from scurvy were found by Findlay (1921) to be comparatively slight. Loss of striation of the muscle fibres, some œdema of the interstitial connective tissue, and in some cases definite hæmorrhagic infiltration of the heart wall were found, but no fatty degeneration or hypertrophy were observed.

Höjer (1924), however, confirmed the findings of the earlier workers, some atrophy, sometimes combined with necrosis, the necrosis having a tendency to go on to calcification.

(c) THE BLOOD VESSELS.—Definite pathological changes, sufficient to account for the typical hæmorrhages of scurvy, have been described by Findlay in the capillaries and smaller venules.

These changes consisted of :

(1) Degenerative changes in the living endothelium, both fatty and granular in character.

(2) Extreme congestion, often to such a degree as to lead to considerable dilatation of the capillaries. As the result of pressure, degenerative changes were not infrequent in the parenchymatous cells of the liver and kidney.

(3) Fine oedema of the tissues surrounding the vessels. This condition was especially observed in the lungs, kidneys, and heart, and appeared to be due to increased transudation of fluid through the damaged capillary wall.

(4) Hæmorrhagic areas in close relation to the capillaries. No actual breach in continuity could be detected in the capillary walls, but Findlay suggests that the red blood corpuscles escape by diapedesis through the intercellular cement substance, whose permeability has been increased by degeneration and swelling of the endothelial cells. A deficiency of intercellular substance is also regarded by Wolbach and Howe (1926) as the essential lesion of scurvy.

Randoin and Michaux (1929) consider that the hæmorrhagic lesions associated with scurvy are due not only to the increased permeability of the capillaries but to an increasing dilution of the blood. They showed that in guinea-pigs on a synthetic vitamin C-free diet the water content of the blood remained normal during the first three weeks, and then began to rise rapidly (from 80 to 91 per cent.) up to the time of death (28 to 32 days).

(d) THE BLOOD—(1) *The Red Corpuscles*.—There appears to be some discrepancy in the red cell counts reported by different observers, but the opinion of the majority is that the alterations, if any, are slight and transient.

A secondary anæmia, with the characteristic decrease in red cells, hæmoglobin, and colour index, has been reported by Meyer and McCormick (1928), and also an increase in reticulated and nucleated red cells. The latter finding, together with polychromatophilia, anisocytosis, and poikilocytosis has been confirmed by Hanke and Koessler (1928).

Findlay, however (1921), while reporting a decrease in the red cells and the hæmoglobin of the blood from the heart, found practically no change in the capillary blood, and stated that nucleated red cells and poikilocytosis were of rare occurrence. He accounts for the discrepancy in the two sources of blood by the capillary stasis and fall of temperature in the later stages of guinea-pig scurvy.

Gasperi (1926) also observed a slight transient decrease in the number of red cells, with slight polychromatophilia, but no marked change in form, hæmoglobin, or granular value.

No appreciable change in red corpuscles and no appreciable diminution in hæmoglobin content was found by Hryniewicz and Lawrynowicz (1927).

Resistance of Red Corpuscles.—An increased resistance of the red cells has been found by Sartori (1928), Meyer and McCormick (1928), de Mare and Brancato (1929), and Randoin and Michaux (1929). The latter workers found that in the normal guinea-pig hæmolysis of the red cells began in solutions of 0.4 to 0.55 per cent., whilst in the scorbutic animals it began in 0.45 to 0.65 per cent. solutions. In the normal animal complete hæmolysis occurred at dilutions varying from 0.3 to 0.2 per cent., but in the scorbutic animal after 3 weeks the resistance of certain of the red cells increased greatly so that by the thirtieth day total hæmolysis only occurred in 0.05 per cent. solution. They attribute the increased resistance to the number of young red cells which have entered the blood in an attempt to replace those lost in the characteristic hæmorrhages occurring in the last stages of the disease.

(2) *The White Corpuscles*.—The changes in the leucocytes are similarly slight and variable. A slight increase in small lymphocytes has been reported by most workers, including de Mare and Brancato (1929), Gasperi (1926), though Meyer and McCormick (1928) found a relative decrease. The leucocytes have been found below normal by Werkman and co-workers (1923), de Mare and Brancato, and Gasperi, and above normal by Meyer and McCormick. It has been suggested by Findlay, however, that the absolute increase in polymorphonuclear leucocytes noted by Meyer and McCormick may possibly be due to a bacterial infection super-

imposed upon the vitamin deficiency. An increase in the number of basophil and eosinophil cells has been observed by Soldani (1929), and Gasperi (1929), and both these workers report that with the Sudan III stainfat droplets could be demonstrated in the large mononuclears.

The Arneth index, according to Werkman and co-workers (1923), shows no significant change. In five normal guinea-pigs the index was 52·5, in 14 scorbutic 56·5.

(3) *Coagulation Time*.—No alteration in the coagulation time has been found by Findlay (1921), Hryniewicz (1927), Nowodworski (1928), confirming the results of earlier workers such as Macrae (1908), etc.

The fibrinogen content of the blood was, however, observed by Jackson and Harley (1900) to be increased (0·76 per cent. as compared with 0·52 per cent. in the monkey), and this result has been confirmed by Randoin and Michaux (1929). The latter workers, using guinea-pigs, state that the fibrinogen content increased gradually from the sixteenth day until death, rising from the normal figure of 1·75 to 3 grms. per 1000 grms. of blood to nearly 6 grms. immediately prior to death.

(4) *Calcium Content*.—A reduction of the calcium content of the blood in human scurvy has been observed by Abraham (1928) and Nowodworski (1928), but Randoin and Michaux (1929) have been unable to confirm this finding with guinea-pigs. According to the latter workers no significant alteration occurred in the blood calcium content, which remained around 130 mgrms. per 1000 c.c. of blood serum.

(5) *Blood Ferments*.—The changes in the enzymes of the blood have been studied by Normark (1924).

Amylase follows the sugar content.

Catalase is increased during the first few days.

Protease and esterase remain normal until the third week, then protease increases and esterase decreases.

Peroxidase shows a primary decrease followed by a return to normal.

(6) *Immunologic Properties*.—According to Werkman, Nelson, and Fulmer (1924), although the resistance of experimentally infected scorbutic guinea-pigs is definitely reduced for pneumococcus and *B. anthracis*, the production of specific agglutinins and opsonins for the typhoid bacillus is not impaired and the phagocyte ability also remains normal.

(e) **THE BONE MARROW**.—In acute scurvy, many small hæmorrhages occur in the bone marrow, followed, according to Randoin and Simonnet (1927), by a gelatinous and fibrinous degeneration of the hæmopoietic cells. It is pointed out by Findlay (1921) that from the evidence of the appearances of the red cells there is no marked erythroblastic reaction of the bone marrow. In chronic scurvy, the bone marrow undergoes a definite gelatinous degeneration and a fibrotic change which is not confined to the epiphyseal ends of the bones (Findlay, 1923).

(f) **IN THE TEETH**.—A characteristic change in structure of the teeth was noted by Jackson and Moore (1916), and was later shown by Zilva and Wells (1919) to be one of the earliest symptoms of scurvy.

A detailed investigation of these changes was made by Höjer (1924). He showed that the changes take place in the dentine, the predentine, and particularly in the odontoblast layer. In a cross-section of the normal incisor stained with hæmatoxylin and eosin, the dentine appears as a broad uniformly-stained layer, the predentine as a narrow white uncalcified portion, and the odontoblasts as a layer of long, slender parallel cells. The pulp tissue consists of small star-shaped cells.

In the tooth of a guinea-pig suffering from fully developed scurvy the dentine is thin, becoming replaced in time by a new formation of spongy porous bone, its uniformity of staining having disappeared. The predentine is calcified and therefore deeply stained.

The odontoblasts have become irregular, the layer narrower, and are in fact, as Höjer emphasises, no longer odontoblasts but osteoblasts, forming bone in any irregular network.

The changes in tooth structure may therefore be summarised as follows :

- (1) Gradual change of odontoblast layer.
- (2) Amorphous calcification of predentine.
- (3) Widening of Tomes' canals in the dentine formed before the onset of scurvy.
- (4) New formation of spongy and porous bone instead of dentine.
- (5) Dilatation of blood vessels, and in the early stages, hyperæmia, sometimes accompanied by hæmorrhages into the pulp.
- (6) Atrophy and resorption of pulp tissue, pulp cells, and newly-formed bone and old dentine.

(g) **THE MUSCLES.**—Minute hæmorrhagic areas in the muscles at the back of both knee joints were found microscopically by Findlay (1921) in guinea-pigs killed after 12 days on a scorbutic diet. Extreme waxy degeneration of muscle has been stated to occur by Meyer and McCormick (1928) and degeneration of the intercostal, masseter, and diaphragmatic muscles has been demonstrated by Dalldorf (1929).

(h) **IN THE ENDOCRINE ORGANS.**—Although the occurrence of widespread minute hæmorrhages is very characteristic of scurvy, it has been pointed out by Findlay (1921) that the endocrine glands, with the exception of the adrenals, show a somewhat remarkable immunity from these hæmorrhages.

(i) *The Adrenals.*—A detailed study of the changes in the adrenals in scurvy was made by McCarrison in 1919, and one of the most important conclusions reached as a consequence of his investigation was that the changes occur before clinical evidences of scurvy manifest themselves.

Macroscopical Changes.—Increase in size, congestion, a reddish-yellow tinge in place of the normal yellow colour, and minute subserous hæmorrhagic effusions were the chief changes observed. The weight was nearly double that of the adrenals of healthy guinea-pigs (0.955 gram average scorbutic, as compared with 0.467 gram average normal).

Histology.—The chief changes consisted of :

- (1) Hæmorrhagic infiltration—more or less circumscribed scattered areas in the cortex.
- (2) Degenerative changes.

In the Cortex these consisted in loss of the tessellated appearance of the cortical cells, vacuolation, and disintegration and disappearance of staining reactions of their nuclei.

In the Medulla, disorganisation and disintegration and loss of the chrome-staining granules of the medullary cells. These cellular changes were not so well marked in the observations of Findlay (1921) except in those animals which had passed into the state of unconsciousness preceding death. Höjer (1924) found a simple atrophy in connection with an early hyperæmia.

Hypertrophy of the adrenals and hæmorrhages were also noted by Morelli and Gronchi (1927). These workers noted two distinct varieties of cellular change, one "progressive" and the other "retrogressive." The "progressive" changes were those which denoted increased glandular activity—increase of siderophil cells, of fuchsinophil granules, and of lipoids, also hyperplasia of the cortical cells with karyokinesis. The "retrogressive" changes were shown by focal hæmorrhages, cloudy swelling, and nuclear degeneration (pyknosis, chromatolysis, karyorrhexis, etc.).

Change in Adrenalin Content.—According to McCarrison (1919) the total adrenalin content, as determined by the method of Folin, Cannon, and Dennis (1912, 1913), showed a pronounced reduction.

Change in Cholesterol Content of Adrenals.—According to Morelli, Gronchi, and Bolaffi

(1928) the cholesterol present in normal adrenals diminishes progressively after an initial increase in scurvy. These workers state that cholesterol neutralises a substance of the nature of "lysocithin"—a derivative of lecithin. The decrease in cholesterol therefore means an increase of "lysocithin," which has a toxic influence on the capillaries. This disturbance, due to the lack of vitamin C, of the metabolism of lipoids regulated by the adrenal cortex may therefore be the direct cause of the hæmorrhages characteristic of scurvy.

(ii) *The Thyroid*.—According to Findlay, hæmorrhages into the thyroid are rare. Hyperplasia and hypersecretion were observed by Morelli and Gronchi (1927).

Similar changes in the thyroid have been observed by Harris and Smith (1928): an increased amount of interfollicular cells, decrease in colloid, increase in number of vacuoles, and lengthening of the cells lining the follicles; the degree of change depending on length of time the animals survive the scurvy-producing diet. Acute starvation (1 c.c. orange juice and water daily) revealed no changes in the thyroid by the method employed. The effect of thyroidectomy in rendering guinea-pigs more susceptible to scorbutic diets was noted by Abderhalden in 1922. Morelli and Gronchi (1927) also state that in thyroidectomised animals the onset of symptoms was delayed, but when they did appear they came on suddenly and with such severity that a return to full diet had no ameliorating effect.

(iii) *The Testes*.—The changes in the testes of guinea-pigs in the early stages of scurvy have been studied by Medes (1926). The changes consisted of engorgement of the blood vessels with degeneration of the seminal epithelium in some of the tubules. Other tubules were normal and contained all stages of developing spermatozoa. Guinea-pigs kept for 30 to 40 days in a state of severe chronic scurvy showed almost complete recovery of the germinal epithelium after 17 days on an antiscorbutic diet.

(iv) *The Spleen*.—Hæmosiderosis, with hæmorrhage and atrophy of the lymphoid tissue, have been the chief changes reported, but Hess (1920) summarised the essential findings by saying "The spleen shares in the general congestion." Slot (1928) reports a hyperplasia of the endothelial cells of the spleen and signs of an increased destruction of red cells. He states that the iron content was considerably increased.

According to Randoin and Michaux (1928) the volume and weight of the spleen increase during the hæmorrhagic stages of scurvy. They state that in the later stages of the disease the cholesterol content and fatty acid content of the spleen diminishes, while the water content increases slightly.

(v) *Other Lymphoid Tissue*.—Höjer (1924) reports that changes in lymphoid tissue are constantly present and resemble those observed in the spleen.

(i) *IN THE ALIMENTARY CANAL*.—An investigation carried out by McCarrison (1919) showed that the chief change occurred in the duodenum, which was intensely congested and swollen, the swelling being due largely to hæmorrhagic infiltration of all coats of the wall. On the inner surface, ecchymoses and areas resembling punched-out ulcers were occasionally found.

McCarrison also described degenerative changes in the myenteron and myenteric plexus. In other parts of the intestine he found marked thinning with occasional occurrence of localised destructive changes leading to ulceration. Slight hyperactivity of the duodenum, measured by means of levers attached to the excised strips, has been reported by Plummer (1927), and, in general, increased tonus of the intestinal wall in scurvy. Smith (1927) found no significant impairment of the motility of the gastro-intestinal canal.

(j) *THE LIVER*.—The changes in the liver in scurvy were summarised by Hess (1920) as being of not sufficient regularity to warrant their acceptance as a distinct lesion, though other workers, including Sato and Nambu (1905), Aschoff and Koch (1919) and Meyer and McCormick (1928), have reported the occurrence of hyperæmia, hæmorrhages, fatty infiltration, and

deposit of pigment. Höjer (1924) found a slight simple atrophy sometimes going on to necrosis.

Iron Content.—Slot (1928) states that the iron content of the liver is considerably increased.

Cholesterol Content.—In the experiments of Randoin and Michaux (1928) the liver content of cholesterol appeared to increase slightly during the course of the disease, though the individual variations were very considerable. The fatty acid content of these livers increased to 3 per cent. during the later stages of the disease.

(k) **THE KIDNEYS.**—The kidneys, according to Hess (1920), are often normal in scurvy, but sometimes show evidence of nephritis with cloudy swelling, congestion, or hæmorrhage. Höjer (1924) found a certain amount of atrophy with a tendency to deposition of calcium.

(l) **THE BRAIN.**—The findings of Findlay (1921) and Meyer and McCormick (1928) are not in agreement on the subject of brain lesions in scurvy. Findlay states that in adult guinea-pigs, hæmorrhage into the central nervous system was never observed, though hæmorrhages in the meninges were quite common.

Meyer and McCormick, on the other hand, report the occurrence of hæmorrhages in the brain, spinal cord, posterior root ganglia, and nerve trunks, also vacuolation in cord and brain, and degenerative changes in central, peripheral, and sympathetic nervous system. Findlay, however, found hæmorrhagic foci into the substance of the medulla and cord in foetuses of guinea-pigs on a scorbutic diet. The total nitrogen of the brain is stated by Palladin and Sswaron (1928) to be increased in scurvy, the water content increased, and the P. content decreased, while the creatin content was practically normal. The P. content was found to be less in scurvy than in starvation.

LXII. FURTHER PHYSIOLOGICAL ASPECTS OF VITAMIN C DEFICIENCY.

(A) **LOWERED RESISTANCE TO INFECTION.**—The question of a possible relation between vitamin C and bacterial infection has been investigated for a fairly large variety of bacteria. The results have been somewhat conflicting, but one interesting theory has emerged from them—that antiscorbutic substances may be developed by the growth of micro-organisms in the tissues. This theory was originated by Jaffe in 1927. He found that animals on a scorbutic diet, when injected with a *Staphylococcus pyogenes albus*, showed slighter symptoms of scurvy than animals on a similar diet and receiving no infection. This observation has been recently confirmed by Baj (1930), who found that the characteristic bone changes of scurvy were less marked in animals infected with staphylococcus, were less predominant than phenomena of suppuration. He, like Jaffe, thinks that the protection may have been afforded by the formation of antiscorbutic substances.

(1) **Common Pathogenic Organisms.**—The general result of investigations into the effect of vitamin C deficiency on infection by pneumococcus, staphylococcus, streptococcus, and *B. coli* is that resistance is somewhat but not markedly lowered.

Findlay (1923), working with pneumococcus, *Staphylococcus aureus*, *Streptococcus hæmolyticus*, and *B. coli* showed that guinea-pigs fed on a diet deficient in vitamin C succumbed to a smaller infecting dose of bacteria than animals fed on a complete diet. He suggested that one of the factors in the reduction of resistance might be the degeneration in the hæmopoetic bone marrow, which he had already shown to be present in experimental scurvy.

Werkman, Nelson, and Fulmer (1923) also found the resistance to pneumococcus definitely though not markedly reduced. They suggested that the increased susceptibility might be an indirect function of the reduced body temperature occurring in avitaminous animals, resulting in depressed phagocytic and bactericidal activity and in favourable cultural environment

in animals normally possessing a body temperature about 37° C., the temperature most favourable for the development of the common pathogenic organisms.

(2) *B. Anthracis*.—Werkman and co-workers found a distinct reduction of resistance to anthrax in scurvy.

(3) *Common Intestinal Bacteria*.—Infection by a mixture of pneumococci and pasteurella organisms was found by Schmidt, Weyland, and Koltsch (1927) to produce severe symptoms in guinea-pigs on a scorbutic diet, while guinea-pigs on a normal diet scarcely ever succumbed to these infections.

A reduced resistance of the bowel wall to the invasion of bacteria and their toxins has been also reported by Mackie and Chitre (1928) to be a definite effect of vitamin C deficiency.

In the course of an investigation of sprue, monkeys were placed on a vitamin C-deficient diet and fed infected material (fæces or monilia therefrom) from cases of this disease. Definite signs of scurvy appeared in most cases, together with marked changes in the large intestine (which were only observed at autopsy and gave rise to no clinical signs), varying from mild inflammation to a condition indistinguishable from ulcerative and sloughing dysentery. Since these changes appeared just as profoundly in the control-deficient animals, which had not received infected sprue material, Mackie and Chitre concluded that they were due solely to the vitamin deficiency. The intestinal changes could not be attributed to amœbæ or dysentery bacilli nor to monilia. In some cases the living tissue of the intestinal wall was found to be invaded by bacteria which are normal inhabitants of the bowel. If the morbid condition had not reached an advanced stage, recovery of the monkeys could be induced by feeding orange juice.

(4) *Tuberculosis*.—It would seem that deficiency of vitamin C does not diminish the resistance to tuberculosis to the same extent as vitamin A. Nor, according to Bloch (1928), does an increased supply of vitamin C have the same beneficial effect as increased vitamin A, except in the rare cases in which the tuberculosis is complicated by scurvy. Some workers, however, including Höjer (1924) and Schutze and Zilva (1927), have found guinea-pigs definitely more susceptible to infection by *B. tuberculosis* when placed on a diet deficient in vitamin C. According to Mouriquand, Rochaix, and Dosdat (1925), the effect of a dietary deficient in vitamin C appears to depend to a certain extent on the dose of bacilli injected. With a moderate number the scorbutic animals exhibit a preliminary phase during which they are more resistant than normal animals; after 3 weeks this resistance suddenly fails, and the disease goes apace. When only a small number of bacilli are given, the disease spreads more rapidly from the start in the scorbutic animals.

(B) **LOWERED RESISTANCE TO TOXIC SUBSTANCES**.—According to Vercellana (1928), guinea-pigs suffering from C avitaminosis are much more sensitive to subcutaneous injections of drugs, such as strychnine nitrate and of aqueous extracts of poisonous fungi, than controls kept on normal diet.

(C) **SENSITIZATION TO A SECOND DEFICIENCY OF VITAMIN C**.—Mouriquand, Michel, and Bernheim (1924) carried out an interesting experiment to determine whether animals which have recovered clinically and anatomically (macroscopically) from deficiency diseases are immunised or sensitised to a second deficiency of the same type.

A series of guinea-pigs was kept for 24 days on a vitamin C-deficient diet, at which time all showed typical scorbutic lesions. They were then kept on a complete diet for from 10 to 20 days or until complete recovery had taken place, after which they were again placed on the deficient diet. At the same time control guinea-pigs, which had been maintained on a complete diet, were also given the scorbutic diet. Among the latter animals the first signs of scurvy did not appear until about the fifteenth day, while in the others, previously cured of scurvy, the symptoms appeared in from 2 to 5 days. The survival period of these animals

was also much shorter than of those suffering from scurvy for the first time. The cause of the increased sensitivity to the effects of the scorbutic diet is thought to be the persistence in the bone marrow of a fibrillar condition which was the only abnormal appearance of the bony tissue of the animals recovered from scurvy.

(D) CONGESTION OF THE BLADDER.—Congestion of the mucous coat and epithelial lining of the bladder without clinical evidence of hæmaturia was described by McCarrison, in 1919, as a consequence of diets deficient in vitamin C. Of the guinea-pigs in which this condition was found only one showed clinical signs of scurvy, so that McCarrison was inclined to regard it as a prescorbutic process, affording an explanation of the comparatively frequent occurrence of hæmaturia in human scurvy.

Swelling of the tissues around the neck of the bladder, engorgement of the vessels with subperitoneal ecchymoses, and congested and ecchymotic areas of the mucous membrane were visible to the naked eye.

The histological changes consisted in—

(1) Congestion of all coats, with hæmorrhagic infiltration of the mucous membrane; extravasation of blood corpuscles into the submucous and muscular coats and between the epithelial cells of the mucous membrane was an almost constant feature. In the more pronounced degrees of hæmorrhagic infiltration, blood corpuscles were extruded into the cavity of the bladder in larger numbers or in such quantity as to constitute actual hæmorrhages.

(2) Degenerative changes in the epithelium of the mucous membrane.

These consisted in—

(1) Swelling of the epithelial cells.

(2) Swelling of the nuclei of these cells and loss of their staining characters.

(3) Desquamation of degenerated epithelial cells.

McCarrison has recently (1930) emphasised the importance of these findings as constituting a predisposition to cystitis.

(E) RETARDATION OF HEALING OF TISSUES.—(1) **Fractures.**—According to Israel and Frankel (1926) and Schilowzew (1928), experimental C avitaminosis retards the healing of fractures considerably.

Israel and Frankel stated that with no vitamin C in the diet fractures in guinea-pigs did not heal. With a limited quantity a condition of pseudoarthrosis was sometimes produced. Both these workers and Schilowzew found that a diet deficient in vitamin C led to spontaneous refracture of a newly healed fracture, and that a diet rich in vitamin C caused rapid and complete recovery.

(2) **Wounds.**—Perroncito (1926) states that vitamin C is amongst the factors, including high oxygen tension, endocrine glands, etc., which promote the rate of regeneration and cicatrisation. With a diet poor in vitamin C, therefore, the healing of wounds will be retarded.

This conclusion confirms the experience of Hehir (1919), who observed that, during the siege of Kut, scurvy had a bad influence on the healing of wounds, especially extensive compound gunshot fractures and large suppurating surfaces.

(F) PATHOLOGICAL LESIONS IN THE FŒTUS.—Under conditions of partial deprivation of vitamin C, Reyher and Walkoff (1928) have observed ill-effects on the fœtus of guinea-pigs. Death or premature birth of the fœtus frequently occurred, or if the offspring survived its resistance was impaired.

Similar pathological and histological features to those encountered in animals were observed in the case of a human fœtus, the mother during pregnancy having received inadequate supplies of vitamin C.

(G) NON-INFECTIVE RHEUMATOID CONDITIONS.—In 1918 Schulhof reported a condition resembling subacute or muscular rheumatism in repatriated prisoners from Russia whose

diet had been notably deficient in antiscorbutic substances. Many of these cases had suffered from the ordinary manifestations of scurvy, but when placed under improved conditions of dietary the hæmorrhagic tendencies disappeared, leaving pains in the limbs and joints which Schulhof considered due to C avitaminosis.

LXIII. VITAMIN C IN CERTAIN FOODSTUFFS.

One of the outstanding characteristics of the distribution of vitamin C in fruits is the fact that though fruit juices are one of its richest sources, their content of vitamin C is by no means equal. Lime juice and grapes, for instance, have been found very inferior in antiscorbutic potency to orange juice and lemon juice. Fruit juices and raw vegetables remain the sources *par excellence* of vitamin C. In 1919 Hehir, as a result of his experience of scurvy during the siege of Kut, expressed the opinion that "all harmless green stuffs and herbs" contained the antiscorbutic principle. As soon as wild herbs and shrubs, including grass, sprang up in sufficient quantity to be collected, cooked, and eaten, the disease incidence declined.

According to Broewer (1927), 1 gram daily of fresh grass (*Lolium perenne*) completely protected guinea-pigs from scurvy for 1 month on a scurvy-producing diet.

(A) **VEGETABLES.**—(1) **Beans, Peas, and Lentils.**—Beans and lentils are not a good source of vitamin C in the dry condition. When sprouted (Santos, 1921; Cohen and Mendel, 1918, etc.), however, they become an excellent source. This was originally shown by Fürst (1912) but has since been confirmed by Delf (1919), Miller and Hair (1918), Simonik (1929), etc. The latter state that the protective dose of mung bean sprouts lies between 3 and 4 grms. Cooked bean sprouts have an antiscorbutic value of approximately 150 units per lb., as compared with lemon juice, orange juice, and tomatoes, 150 to 300 units, on Sherman's basis.

STRING BEANS are a fair source (about 112 units per lb.), according to Quinn, Burtis, and Milner (1927).

Eddy (1929) has found canned string beans adequate in a dosage of 18 grms. per day by Sherman's method, but was not able to find a dose by Höjer's method which could be used without too great a reduction of other factors in the basal intake.

GREEN PEAS.—According to Eddy and co-workers (1926), green peas are a rich source of vitamin C, equivalent to tomatoes or oranges. Young peas are more potent than old, maturity resulting in a reduction of vitamin C content.

CANNED PEAS.—Eddy states that canning has no marked destructive effect on the vitamin C content of peas. Blanching is more destructive than processing, and reheating the peas in an open kettle does not reduce the potency as much as ordinary cooking.

(2) **Cabbage.**—The raw leaf tissue of cabbage is one of the most potent sources of vitamin C. Delf (1918) estimated the daily minimum protective dose at 1 gram.

Cooking and preservation reduces its potency considerably, 15 grms. being necessary after cooking for 1 hour at 100° C. (Delf), and something less than 50 grms. after drying (Shorten and Roy, 1921), and 7.5 grms. after canning (Campbell and Chick, 1919).

Eddy and co-workers, however (1923, 1924), state that canned cabbage has a much greater potency than home-cooked, 4 grms. daily of the canned variety being equivalent to 20 of the home-cooked. They suggest that this higher potency may be due either to the fact that canned cabbage is freshly harvested and has therefore undergone no loss through storage, or to the exclusion of oxygen during the canning process.

A cabbage soup and a cabbage purée have been used by Tso (1928) as an inexpensive source of vitamin C for infant feeding.

Experiments with guinea-pigs showed that 30 c.c. of the soup and 5 to 10 c.c. of the purée were the minimum daily protective dose.

A powder extracted from fresh cabbage in the absence of oxygen (according to the method of Bezssonoff) is stated by Rohmer (1929) to have a specific therapeutic action in scurvy, and to produce no digestive disturbance.

Sauerkraut, prepared from cabbage by fermentation, has been found by Clow and co-workers (1929) to be completely protective against scurvy in a daily dosage of 10 grms. This amount appeared to be equivalent in vitamin C to 5 grms. of raw stored cabbage. These workers point out that oxidation due to exposure in repacking and retailing is responsible for loss of potency rather than fermentation, so that commercial sauerkraut may not be so potent as the above experiment would imply. In their later experiments (1930) they found considerable variation in the potency of six brands tested.

(3) **Carrots.**—Raw young carrots have, according to Hess and Unger (1919), a higher antiscorbutic potency (10 grms. daily as the minimum protective dose) than old raw ones which vary from 10 to 35 grms. This juice is potent in a dosage of 20 c.c. (Chick and Rhodes, 1918). As with cabbage, cooking, drying, and preservation reduce the antiscorbutic potency of carrots.

(4) **Cauliflower, etc.**—Cauliflower (boiled for $\frac{1}{2}$ hour), is protective in a dosage of 30 to 60 grms., dandelion greens 30 grms., endive and lettuce 15 grms. (Holst and Frölich, 1912).

(5) **Marrow and Pumpkins.**—The estimation carried out by Wats (1929) gives a higher degree of potency to pumpkin than that of Delf (1921). Delf found 13 to 20 c.c. of juice equivalent to 75 to 100 grms. of pulp, the minimum protective dose, while Wats states that 10 grms. of pumpkin and 15 grms. of vegetable marrow are sufficient.

Delf gave 12.5 grms. for young marrow pulp, more than 37.5 grms. for matured. From these results Wats estimates that $3\frac{1}{2}$ ounces net of pumpkin or melon pumpkin and over 5 ounces net of vegetable marrow will be required per man per day. It must be remembered, however, that a considerable portion of these vegetables is thrown away during the process of peeling.

(6) **Potato.**—According to Givens and McClugage (1920) the cooking of potatoes, even baking, reduces their potency only slightly unless they are cooked for an hour, but the potato is not a very valuable antiscorbutic, its potency being only $\frac{1}{6}$ to $\frac{1}{10}$ that of cabbage.

(7) **Peppers.**—A high concentration of vitamin C in green peppers has been reported by Quinn, Burtis, and Milner (1927). They state that they contain at least four times as much vitamin C as string beans, or about 1 unit per gram. This concentration is comparable with the highest which has been reported for any plant material, such as the raw leaf tissue of cabbage or spinach or the juice of orange or lemon.

(8) **Turnips.**—The white turnip and the mangold have approximately the same antiscorbutic potency, of a rather low degree, about 40 grms. of mangold (Boock and Trevan, 1922) and about 50 grms. of turnip (Shorten and Roy, 1921) being the minimum protective dose. Swede juice, however, has a high potency, 2.5 c.c. according to Chick and Rhodes (1918) being sufficient. Turnip juice, from the Chinese winter turnip, was found by Tso (1928) to be necessary in a dosage of 20 c.c.

(9) **Watercress.**—According to Coward and Eggleton (1928) watercress is of high potency, equal approximately to that of cabbage, 1 gram daily being sufficient to protect guinea-pigs against scurvy for a period of 70 days.

(B) **FRUITS.**—All the fresh citrous fruits have long been recognised as good sources of vitamin C, but lime juice has now been proved to be of low potency compared with lemon juice.

(1) **Lemons.**—So generally has the antiscorbutic potency of lemons been recognised that after the compulsory issue of lemon juice to the Navy in 1804 scurvy became a

comparatively rare disease. The investigations of Zilva (1924–1928) have been based upon this property of lemons, the juice having been used as the original source of his attempts to isolate vitamin C.

According to Davey (1921) whole lemons can be kept at 2° to 5° C. for 5 to 6 months without injuring the potency of their juice. At room temperature in presence of rind oil lemon juice retains its potency, and at 0° C. in presence of 0.06 to 0.1 per cent. of potassium metasulphite, though the effect of this agent is uncertain at room temperature. Certain varieties of lemons newly introduced into the East Indies have been examined by Jansen and Donath (1925), as it seemed important to determine whether they belonged to the powerfully antiscorbutic "lemon" class or to the much less effective "lime" class. The *Citrus vulgaris* imported from Brazil and the sweet orange from Surinam contain very little vitamin C, 10 c.c. a day being necessary to protect guinea-pigs from scurvy. Similar figures were obtained for the *Citrus lemonellus*, a species of lime grown in the East Indies for the preparation of lemon squash. On the other hand, the *Citrus nobilis*, a sweet orange resembling our tangerine, and the *Citrus decumana*, both grown in the East Indies, contain at least twice as much vitamin C as the three varieties first mentioned. Samples of certain commercial lemon and lime juices were also tested, and it was found that 15 c.c. were not a certain protection against scurvy.

LEMON RIND.—According to Willimott and Wokes (1926) the rind of fresh lemons contains only small amounts of vitamin C.

TABLETS OF DRIED LEMON JUICE.—Tablets have been prepared by Bassett Smith (1920) each corresponding to about half a fresh lemon in antiscorbutic potency. The lemon juice is evaporated over H_2SO_4 at 3.5° C. to 15.5° C., and the syrup mixed with lactose and gum tragacanth.

(2) **Oranges.**—Oranges are of about the same antiscorbutic potency as lemons. Like lemon juice, orange juice retains its antiscorbutic property at low temperatures for long periods. By an Order in Council (Statutory Rules and Orders, 1927, Merchant Shipping), provision was made regarding the use of orange juice as an alternative to lime or lemon juice as an antiscorbutic. The concentrated orange juice used was to contain not less than 70 per cent. of total solids by weight. It must show no signs of alcoholic fermentation, and must contain no mould growths. It must be prepared and stored so that there should be no material loss of vitamin potency. The amount to be given out daily was at the rate of 1½ fluid ounces mixed with 6 times its value of water.

A concentrated preparation of orange juice investigated by Priston (1926) was stated to be potent in a daily dosage of 5 c.c., and to preserve its potency to a marked degree after storage for a year at 8° C.

A method of treating severe cases of scurvy by intravenous injections of orange juice was described by Spencer (1926). Two injections of 10 c.c. of raw orange juice in 150 c.c. of normal saline were given at intervals of 1 week with good results.

Working with guinea-pigs, Eddy (1929) has found the minimum protective dose of orange juice 1.5 c.c. by Sherman's method, 3 c.c. by Höjer's.

ORANGE RIND.—According to Willimott and Wokes (1926) orange rind contains more vitamin C than does lemon rind.

(3) **Limes.**—Lime juice was in the early days of scurvy investigation regarded as a powerful antiscorbutic, but later experiments (Chick, Hume, and Skelton, 1918; Robison, 1919) showed it to be very inferior to lemon juice.

The investigations of Smith (1918, 1919) showed that the lime juice used until the middle of the nineteenth century was really lemon juice, and it was not until West Indian lime juice was gradually substituted for it that its inefficiency in preventing scurvy was discovered.

(4) **Grape Fruit.**—According to MacLeod and Booher (1930) the protective dose of fresh grape fruit is 2 grms. daily, while that of canned grape fruit is 2.2 to 2.8 grms.

(5) **Grapes.**—Grapes and fresh grape juice have been found inferior to lemons and oranges (Chick and Rhodes, 1918), while dried grape juice, according to Givens and Macy (1921), is practically devoid of antiscorbutic potency. Randoin (1928) found the freshly pressed juice of grapes, when fed at the rate of 6 c.c. daily, capable of extending the life of guinea-pigs on a vitamin C-free basal diet from 40 to 60 days. Preparations of concentrated juice, however, containing preservatives were found to possess no antiscorbutic action. White wine was also found to contain very little vitamin C, and this amount disappeared completely on concentrating the wine either in the air or under reduced pressure.

(6) **Apples.**—Apples have until recently been considered a not very good source of vitamin C. Givens, McClugage, and van Horne (1921, 1922) gave 10 grms. of raw apple as an adequate daily dosage, but found dried apples to contain but very little vitamin C. According to Bracewell, Hoyle, and Zilva (1930), however, different varieties of apples vary considerably in their antiscorbutic potency, Bramley's Seedlings being decidedly more active than any others tested. The Dabmett, a cider variety, was next in potency, and Cox's Orange Pippin third. Canadian apples had approximately the same potency as English, but Australian and New Zealand apples were not so potent. The difference in potency of the different varieties appears to be a difference of species and not dependent on the age of the tree or conditions of cultivation.

CANNED APPLES are stated by Eddy and Kohman (1923, 1924) to be more potent than raw stored apples if the canning is carried out with due regard to prevention of oxidation. By covering apples with salt solution to enable their respiratory process to use up their intercellular oxygen they were found to withstand canning with no loss of vitamin C.

(7) **Bananas.**—Various workers have emphasised the value of the banana as a suitable antiscorbutic for infants. Von Meysenbug (1928) states that it is second only to orange juice. The actual optimum quantity has not been entirely agreed upon. Lewis (1919) gave 10 to 15 grms. as the figure, while Givens and McClugage (1922) and Jansen and Donath (1925) also reported that 10 grms. daily was protective.

Eddy and Kellogg (1927), however, using the Sherman and La Mer basal diet fixed the minimum protective dose at 5 grms. By the more sensitive Höjer method, Eddy (1929) found 10 grms. necessary to prevent disordered tooth formation.

Baked bananas, according to Eddy and Kellogg (1927), retain their antiscorbutic factor intact if baked in the skin, whereas without the skin the amount of vitamin C was reduced to one-third its normal value.

(8) **Cranberries.**—According to MacLeod and Booher (1930), cranberries are a poor source of vitamin C, though fresh cranberry was richer than canned cranberry sauce.

(9) **Dates.**—Although definite value were not obtained, MacLeod and Booher (1930) state that they are a poor source of vitamin C.

(10) **Peaches.**—Raw peaches are a good source of vitamin C (Delf, 1921).

According to Eddy, Kohman, and Carlsson (1926) the minimum antiscorbutic dose is about 5 grms. per day. These workers state that raw and canned peaches contain about 5 times as much vitamin C as kettle cooked. The method described for canning apples was not so applicable in the case of peaches. The respiratory bound oxygen is not so evident in peaches as in apples, and therefore elimination of oxygen by exhaustion before canning was not observed to increase their antiscorbutic value.

Dried peaches are reduced in vitamin C content (Eckman, 1922; Morgan and Field, 1929).

Sulphured peaches, however, are stated by Morgan and Field to rank with orange juice, raw tomatoes, and other highly potent antiscorbutic foods.

(11) **Pears.**—According to Craven and Cramer (1927) the vitamin C potency of raw

pears is about one-quarter of that of orange juice, the minimum protective dose for guinea-pigs being from 10 to 15 grms., and storage does not lessen the antiscorbutic factor. The effect of canning is approximately the same as for peaches.

(12) **Prickly pear** (*Cactus*), according to Delf (1921), is a good source of vitamin C.

(13) **Avocado pear** is stated by Dickey (1927) to be insufficient when given with water and oats as the sole diet to prevent scurvy in guinea-pigs.

(14) **Strawberries** are a fairly rich source of vitamin C. According to Kohman, Eddy, and Halliday (1928), canned strawberries are as rich as canned tomatoes, and their potency is not destroyed by canning.

(15) **Some Tropical Fruits.**—(a) **PAPAYA** is rich in vitamin C (Delf, 1921; and Miller, 1926) as well as in the ferment "papain" for which its juice is largely used.

(b) "**RED FRUIT**" (*Cratageus pinnatifida*) is, according to Hsu (1928), about one-third as potent as orange juice. He states that subjecting the syrup to 110° to 120° C. for a few minutes, and allowing it to dry, does not decrease its vitamin C content.

(c) "**HAITANG**" and **PERSIMMON** are poor in vitamin C.

(d) **POMEGRANATE** and **HSIANG TS'AI** (*Coriandrum sativum* Linnæus) are stated by Sherman (1929) to be rich in vitamin C.

(e) **CUSTARD APPLE.**—According to Schlossberg (1929), cherimoya (*Anona cherimolia*) [a tropical fruit of the custard-apple type] afforded protection from scurvy for 3 months in daily doses of 15 to 20 grms. With doses of 5 to 10 grms. a mild form of scurvy developed in 28 to 50 days.

(f) **PIMENTO.**—According to MacLeod and Booher (1930), canned pimento has about the same antiscorbutic value as grape-fruit, the protective dose being 2 grms. daily.

(C) **ANIMAL TISSUES.**—The vitamin C activity of animal tissues is on the whole low.

(1) **Beef.**—It is generally recognised that fresh meat is very low in antiscorbutic potency, though the experience of the pioneers of sea-voyaging seems to show that it had some preventive action against scurvy. Hehir (1919) states that "fresh meat" alone without vegetables will not indefinitely postpone scurvy; it seems to delay its appearance.

Medes (1926) observed a not very definite result of a delay in the development of scurvy in guinea-pigs consuming 20 to 30 grms. of meat daily.

(2) **Liver.**—According to Kaneko (1927), liver is richer in vitamin C than muscle, heart, cerebrum, or kidney. A water extract of fresh liver, prepared by pounding 60 grms. of liver pulp with 60 c.c. of water in a mortar, allowing it to stand, and then filtering, is stated by Aron, Hirsch-Kauffmann, and Schadrach (1928) to prevent scurvy in guinea-pigs. They suggest that as a prophylactic agent in infant feeding this extract might be better tolerated than fruit juice.

A therapeutic effect of liver in scurvy has been reported by Slot (1930), but he seems to regard it as the effect rather of substitution of deficient liver substances, caused by C avitaminosis, rather than of its content of vitamin C.

(3) **Edible Molluscs.**—According to Marchi (1928), various edible molluscs, of which *Cardium edule* has the highest content, contain vitamin C. Randoin (1923) has also found it present in oysters.

(4) **Eggs.**—Egg-yolk has been found practically devoid of antiscorbutic potency (Hauge and Carrick, 1925). Bezssonoff (1926), indeed, uses it as one of the chief constituents of a scurvy-producing diet. Dougherty (1926), however, found that egg-white apparently delayed the onset of scurvy as compared with egg-yolk.

(E) **MILK**—(1) **Human.**—Human milk has been tested for its antiscorbutic potency with not completely satisfactory results.

Meyer and Nassau (1925) found that although they were unable to keep guinea-pigs alive

on a diet of fresh oats and human milk, the deficiency was not that of vitamin C but of protein. None of the animals which succumbed showed signs of scurvy, and when the diet was supplemented with a protein preparation not containing vitamin C normal growth took place.

Macy and Outhouse (1928) state that normally the amount of vitamin C in human milk is small and is known to fluctuate according to the vitamin C content of the maternal diet.

(2) **Cows' Milk.**—Cows' milk is not a potent antiscorbutic and, moreover, is variable in its vitamin C content. The protective dose, as estimated by different workers, varies from 85 to 130 c.c. (Chick, Hume, and Skelton, 1918) to 20 to 60 c.c. (Dutcher *et al.*, 1920).

The relation between the vitamin C content of the milk and the food given to the cow is not definitely established. Earlier workers, including Barnes and Hume (1919); Hart, Steenbock, and Ellis (1920), Dutcher *et al.* (1920); and Hess, Unger, and Supplee (1920) were agreed that the antiscorbutic potency of milk is greatly reduced when the diet of the cow is poor in antiscorbutic food. Reyher (1926) also found seasonal differences in the vitamin C content, summer milk being richer than winter, while MacLeod (1927) found that a ration containing corn silage, but no fresh pasture, produced milk of high antiscorbutic value irrespective of season. Silage milk is reported by Kieferle and Zeiler (1926) to be of high antiscorbutic potency.

(3) **Boiled Milk.**—Clinical experience tends to show that boiling milk reduces to a large extent its antiscorbutic potency. Heating to 120° for 1 hour was stated by Chick, Hume, and Skelton (1918) and Hart, Steenbock, and Smith (1919) to render it so devoid of potency that it could be used as a constituent of a scurvy-producing diet for guinea-pigs. Bezssonoff (1926), however, suggested that the vitamin C of milk was by no means completely destroyed by autoclaving for 1 hour at 120° C. He found that whereas animals fed on a diet composed of oats, bran, yeast, and an emulsion of fresh egg-yolk required 3 c.c. of lemon juice for protection against scurvy, 1.5 c.c. was sufficient if 60 to 80 c.c. of autoclaved milk were one of the constituents. A more definite statement as to the amount of destruction produced by boiling has recently been made by Schwartz, Murphy, and Hann (1930). They found that the amount of antiscorbutic vitamin destroyed by lightly boiling 3 quarts of milk for five minutes in a glass beaker or in an aluminium stew-pan was approximately 20 per cent.

(4) **Pasteurised Milk.**—Certain outbreaks of infantile scurvy, such as those recorded by Neumann (1902), Heubner (1903), Hess and Fish (1914), and Miller (1917) have been attributed to the practice of pasteurising the milk, but there has not been indisputable evidence that the conditions of heating were always those of pasteurisation only.

According to Cavanagh, Dutcher, and Hall (1924), pasteurised milk retains at any rate some of its antiscorbutic potency.

(5) **Condensed Milk.**—The destruction of vitamin C in condensed milk depends to a large extent upon the process used.

SWEETENED CONDENSED MILK, according to Hume (1921) and Hess (1921), undergoes no appreciable loss of vitamin C. This is attributed to the low temperatures used and the absence of air. The extreme dilution of the milk for infant feeding must, however, produce a serious dilution of its vitamin content.

UNSWEETENED CONDENSED MILK, on the other hand, is subjected to a more drastic treatment. According to Hart, Steenbock, and Smith (1919) the loss of vitamin C is more than 40 per cent., while Hottinger (1927) found that such a preparation afforded almost no protection against scurvy in 100 c.c. amounts.

(6) **Dried Milk.**—If the drying process is carried out rapidly and with comparative absence of moisture, there is apparently no appreciable loss of vitamin C (Cavanagh, Dutcher, and Hall (1924)).

According to Jephcott and Bacharach (1921), and Supplee and Dow (1926), milk dried by

the roller process undergoes less vitamin C destruction than when the spray process is used. Scurvy was prevented in guinea-pigs by 80 c.c. of roller process milk, but not completely prevented by 90 c.c. of spray process milk.

Milk powders tested by Donath (1929) contained little, if any, vitamin C.

(7) **Irradiated Milk.**—According to Reyher (1926), irradiation of raw milk in air destroys its antiscorbutic potency, the destruction being due to the production of ozone. If irradiated *in vacuo* no destruction takes place. A dried sweetened milk examined by Hottinger (1927) was unaffected by irradiation.

(8) **Goats' Milk.**—A lower vitamin C content of goats' milk than of cows' has been indicated by the experiments of Hunt and Winter (1922) and of Meyer and Nassau (1924). The latter workers suggest that this may be a partial explanation of the so-called goats' milk anæmia observed in children fed on this milk.

(D) **GREEN TEA.**—A Japan green tea, labelled "rich in vitamin C," has led to the investigation of the statements of various workers, including Miura and Tsujimura (1924), that green tea would delay scurvy in guinea-pigs. In spite of the tentative suggestion of Miura (1929) that steeping the tea at 70° to 75° C. for 5 minutes may have destroyed about 74 per cent. of its antiscorbutic activity, the conclusions of Mattill and Pratt (1928), Munsell and Kifer (1929), Mitchell (1929), and Munsell and Miller (1930) are all definitely to the effect that Japanese green tea contains no demonstrable amount of vitamin C.

TABLES SHOWING THE VITAMIN CONTENT OF FOODSTUFFS

THE natural distribution of vitamins in foodstuffs has been found to be partial and irregular, and the classification of different groups of foodstuffs, according to the particular vitamin which they contain *par excellence*, cannot be made according to any hard-and-fast rule, since the content of one or other vitamin is very often a matter of degree only. But there are certain lines which can be drawn, mapping out roughly certain areas of distribution which form more potent sources of one vitamin than another.

Generally speaking, foodstuffs may be classified according to their vitamin content as follows :

- (1) Green plants, rich in vitamin A and containing a certain amount of vitamin C and E.
- (2) Oils, animal fats, and milk—sources of the fat-soluble vitamins A, D, and E.
Cod-liver oil is a potent source of vitamins A and D. Butter contains A, D, and E. Vegetable oils, particularly wheat germ oil, are rich in vitamin E.
- (3) Yeast and the growing embryo of cereals—the chief sources of the vitamin B complex.
- (4) Fresh fruits and vegetables—the chief source of vitamin C.

Estimations of the actual value of the various foodstuffs are constantly in progress, but the biological tests are necessarily slow, and until each foodstuff has been examined in detail it is only possible to draw up tables which give a more or less accurate idea of the comparative vitamin content of each article of food. The following tables have been drawn up on the basis of the 1924 *Report* (reprinted in 1928) of the *Medical Research Council*, on Professor Plimmer's tables in his *Handbook on Vitamins*, on the United States Department of Agriculture Circular, No. 84, and Randoin and Simonnet's *La Question des Vitamines*, supplemented with the results of investigations, recently published, into the content of various foodstuffs in particular. The actual amount of vitamins in certain articles of food have been worked out in detail by various observers. These results have been incorporated in the special section devoted to the vitamin under discussion.

TABLES SHOWING THE VITAMIN CONTENT OF FOODSTUFFS

Explanation of signs used:

+++ = very rich
++ = rich

+ = fairly rich
± = some present

- = absent
D = doubtful

Class of Foodstuffs.	Individual Foodstuff.	References.	VITAMIN					
			A	B ₁	B ₂	C	D	E
ANIMAL FOODSTUFFS AND TISSUES.	Bacon	Sherman (1926)	- to +	+				
	Beef Extract ...	Hoagland and Snider (1930)			+ to ++			
	Beef Muscle ...	McCollum, Simmonds, and Parsons (1921)	D					
		Hoagland and Snider (1925)	±					
		Sherman (1926)	±					
		Grijns (1901)		±				
		Cooper (1912, 1914) ...		±				
		Osborne and Mendel (1917)		±				
		Hoagland (1929)		±				
		Goldberger (1928)			+			
		Aykroyd and Roscoe (1929)			+			
		Hoagland (1929)			+			
		Hehir (1919)				- to +		
		Dutcher, Pierson, and Biester (1920)				- to +		
		Medes (1926)				- to +		
		Evans and Bishop (1923)...						+
		Evans and Burr (1927) ...						+
	Brains	Penau and Simonnet (1922)	+					
		Sherman (1926)	+					
		Cooper (1912, 1914) ...		+				
		Osborne and Mendel (1918)		+				
	Buttermilk ...	Drummond, Coward, and Watson (1921)	+					
		Sherman (1926)				- to +		
	(Irradiated) ...	Goettsch and Tolnai (1926)					+	
	Casein	Randoin and Simonnet (1927)	+	+				
		Chick and Roscoe (1928) ...		+				
		Evans and Burr (1928) ...			+			
		Randoin and Simonnet (1927)				-		
	(Leached) ...	Goldberger and co-workers (1930)			+			

Class of Foodstuffs.	Individual Foodstuff.	References.	VITAMIN CONTENT			
			A	B ₁	B ₂	B ₆
ANIMAL FOODSTUFFS <i>continued.</i>	Cheese	Sherman (1926)	++			
	(Whole Milk)	Plimmer (1922)	+			
	(Skim Milk) ...	Plimmer (1922)	—			
	(Cottage, Skim)	Smith (1929)	+			
	American ...	Morgan (1926)	+++			
	Limberger ...	Morgan (1926)	+++			
	Swiss ...	Morgan (1926)	+++			
		Cooper (1912, 1914) ...		< +		
		Sherman (1926)		D		
	Eggs— Ducks' ...	Jansen and Donath (1924)	++			
		Tso (1926)	++			
	Hens' (Dried)	<i>Med. Res. Council Report</i> (1924-8)	++			
		McCollum and Davis (1915)		— to +		
		Chick and Hume (1917) ...		++		
		Tso (1926)		++		
		Chick and Hume (1917) ...				
		Hess and Weinstock (1924)				
	(Fresh)	Murphy and Jones (1925)	++			
		Sherman (1926)	++			
		Chick and Hume (1917) ...		++		
		Osborne and Mendel (1923)		++		
		Sherman (1926)		++		
		Hess and Unger (1918) ...				
	(Irradiated)	Hart and co-workers (1925)				
		Hughes and co-workers (1925)				

Individual Foodstuff.	References.	VITAMIN				
		A	B ₁	B ₂	C	D
eggs (Yolk)— <i>continued.</i>	Palmer and Kennedy (1921)	++				
	Munsell (1924)	++				
	Bethke, Kennard, and Sassamann (1927)	++				
	Steudel (1929)	++				
	Cooper (1912-14)		+			
	Steenbock (1917)		++			
	Osborne and Mendel (1919)		+			
	Goldberger (1928)			++		
	Aykroyd and Roscoe (1929)			++		
	Chick and Hume (1917) ...				—	
	Hauge and Carrick (1925)				—	
	Dougherty (1926)				—	
	Bezssonoff (1926)				—	
	Hess (1923)... ..					++
	Casparis, Shipley, and Kramer (1923)					++
	Tso (1926)					++
	Jex-Blake (1927)					++
	Bethke, Kennard, and Sassamann (1927)					++
	Steudel (1929)					++
	Evans and Bishop (1923)...					
(White) ...	Evans and Burr (1927) ...					
	<i>Med. Res. Council Report (1924-8)</i>	—				
	Vedder (1912)		—			
	Bond (1922)		—			

Class of Foodstuffs.	Individual Foodstuff.	References.	VITAMIN					
			A	B ₁	B ₂	C	D	E
ANIMAL FOODSTUFFS—continued.	Fish—continued. (Haddock)	Kik and McCollum (1928)	+	— to +			+	
	Canned Haddock	Goldberger and co-workers (1930)			+			
	Fat (Salmon, Herring)	Drummond (1918)... ..	++					
	Herring ...	Eddy (1921)		— to +				
		Hartwell (1922)		— to +				
		Kik and McCollum (1928)	+	— to +			+	
		Schmidt-Nielsen (1929) ...	++					
	Eels (Dried) ...	Shimoda, Fujimaki, and Saiki (1927)	++					
	Roe (Cod) ...	McCollum and Davis (1913)	++					
		Zilva and Drummond (1922)	++					
		Hjort (1922)	++					
		Chick and Hume (1917) ...		+				
		Sherman (1926)		++				
	(Herring)	Coward and Drummond (1922)	++					
	Salmon (Canned)	Sherman (1926)	+					
		Goldberger (1928)			++			
		Goldberger and Wheeler (1929)			++			
	Toheroa (Canned)	Malcolm 1929)	+					
	Shell Fish—Clams ...	Jones, Nelson, and Murphy (1928)	+	— to +			+	
	Molluscs ...	Marchi (1928)				+		..
	Oysters ...	Shimoda, Fujimaki, and Saiki (1927)	+					
		Jones, Nelson, and Murphy (1928)	+	++			++	
		Randoin (1923)				+		
	(Canned)	Malcolm (1929)	+					
		Russell, Morrison, and Ebling (1926)					++	
	Shrimps ...	Hjort (1922)	+					
	Ham	Smith (1929)	— to +					
		Hoagland (1929)		++				
	Heart	Osborne and Mendel (1918)	++					

Class of Foodstuffs.	Individual Foodstuff.	References.	VITAMIN					
			A	B ₁	B ₂	C	D	E
ANIMAL FOODSTUFFS—continued.	Heart—continued.	Cooper (1912, 1914) ...		+				
		Osborne and Mendel (1918)		++				
		Sherman (1926) ...				+		
	Kidney ...	McCollum and Davis (1915)	+					
		Osborne and Mendel (1918)	++					
		McCollum, Simmonds, and Parsons (1921)	+					
		Sherman and Boynton (1925)	+					
		Osborne and Mendel (1918)		++				
		Sherman (1926) ...				+		
		Evans and Burr (1927) ...						+
	Lamb or Mutton	Hoagland and Snider (1925)	— to +					
		Sherman (1926) ...		+				
	Liver ...	Osborne and Mendel (1918)	++					
		Sherman and Boynton (1925)	++					
		Osborne and Mendel (1917)		+				
		Mollow (1928) ...			+			
		Kaneko (1927) ...				+		
		Evans and Burr (1927) ...						+
	Ether Extract	Penau and Simonnet (1922)	+++					
	Water Extract	Aron <i>et al.</i> (1928) ...				+		
	Irradiated ...	Steenbock and Black (1924)					+	
	Calf, Ox, Sheep	Rosenheim and Webster (1927)	+++					
	Beef ...	McCollum, Simmonds, and Parsons (1921)	+++					
		Laqueur <i>et al.</i> (1928) ...	++					
		Cooper (1912, 1914) ...		+				
		Osborne and Mendel (1918)		+				
		Goldberger and co-workers (1928)			++			
		Aykroyd and Roscoe (1929)			++			
		Evans and Burr (1927) ...						++
	Birds' ...	Rosenheim and Webster (1927)	+					
	Chicken ...	Hart, Steenbock, and co-workers (1925) ...		+		+		
	Fish (<i>see also</i> Fish Liver Oils)	Zilva and Drummond (1912)	+++					

Class of Foodstuffs.	Individual Foodstuff.	References.	VITAMIN					
			A	B ₁	B ₂	C	D	E
ANIMAL FOODSTUFFS —continued.	Pig	McCollum and Davis (1915)	+					
		Osborne and Mendel (1917)	+					
		Penau and Simonnet (1922)	+					
		Osborne and Mendel (1923)		+				
		Goldberger and co-workers (1928)			+			
		Evans and Burr (1927) ...						+
	Milk— Cows' : Skim (Raw)	Osborne and Mendel (1913)	— to +					
		McCollum and Davis (1915)	— to +					
		Drummond, Coward, and Watson (1921)	— to +					
		Simonnet (1922)	— to +					
		Sherman (1926)	+	+				
		Platon (1927)	Almost nil					
		Johnson and Hooper (1922)				+		
		Sherman, McLeod, and Kramer (1920)	+					
		Johnson (1921)		+				
		Johnson and Hooper (1921)		+				
		Sherman and Spohn (1923)		+				
		Sherman and Axtmayer (1927)			++			
		Smith (1929)				— to +		
		Evans and Burr (1927) ...						— to +
	Cows' : Skim (Dried)	Drummond, Coward, and Watson (1921)	++					
		Sherman (1926)	+++					
		Donath (1929)	++					
		Donath (1929)		— to +				
		Hume (1921)				++		
		Hess (1921)				++		
		Med. Res. Council Report (1924-8)	++	+				
		Hart, Steenbock, and Smith (1919)				— to +		
		Hottinger (1927)				Almost nil		
		Sherman, Rouse, Allen, and Woods (1921)	+++					
	Cows' : Whole Condensed (Sweetened)	Drummond, Coward, and Watson (1921)	++					
		Sherman (1926)	+++					
		Donath (1929)	++					
		Donath (1929)		— to +				
	(Unsweetened)	Hume (1921)				++		
		Hess (1921)				++		
		Med. Res. Council Report (1924-8)	++	+				
		Hart, Steenbock, and Smith (1919)				— to +		
	Cows' : Whole (Dried)	Hottinger (1927)				Almost nil		
		Sherman, Rouse, Allen, and Woods (1921)	+++					

Class of Foodstuffs.	Individual Foodstuff.	References.	VITAMIN					
			A	B ₁	B ₂	C	D	E
ANIMAL FOODSTUFFS —continued.	Milk— Cows' : Whole Dried—contd.	Sherman (1926)	++					
		Johnson (1921)		++				
		Sherman and Spohn (1923)		++				
		Hartwell (1925)		++				
		Sherman (1926)		++				
		Daniels, Giddings, and Jordan (1929)		+ to ++				
		Donath (1929)		— to +				
		Barnes (1919)				— to +		
		Chick and Dalyell (1919)...				— to +		
		Hart, Steenbock, and Smith (1919)				— to +		
		Hess and Unger (1919) ...				— to +		
		Jephcott and Bacharach (1921)				— to +		
		Cavanaugh, Dutcher, and Hall (1924)				— to +		
		Supplee and Dow (1926) ...				— to +		
		Donath (1929)				Almost nil		
		Supplee and Dow (1927) ...					+	
		Anderegg and Nelson (1926)						+
	Cows' : Whole Dried (Irradiated)	Sherman, Rouse, Allen, and Woods (1921)	+++					
		Supplee and Dow (1927) ...	+++			— to +	+++	
		Hottinger (1927)				— to +	+++	
		Hess, H. A. (1925)					+++	
		Mackay and Shaw (1925)...					+++	
		Wieland (1928)					+++	
		Hess and co-workers (1929)					+++	
		Drummond, Coward, and Watson (1921)	++					
	Cows' : Whole (Evaporated)	Dutcher, Honeywell, and Dahle (1927)	+ to ++					
		Dutcher and Francis (1926)		++				
		Sherman and MacArthur (1927)		++				
		Daniels and Brooks (1927)		— to +	++			
		Cavanaugh, Dutcher, and Hall (1924)				— to +		

Class of Foodstuffs.	Individual Foodstuff.	References.	VITAMIN					
			A	B ₁	B ₂	C	D	E
ANIMAL FOODSTUFFS —continued.	Milk— Cows' : Whole (Evaporated) —continued	Honeywell and co-workers (1930)					— to +	
		Dutcher and co-workers (1927)	+++					
		Schmidt-Nielsen (1929) ...	— to +					
		Johnson (1921) ...		++				
		Daniels and co-workers(1929)		—to++				
		Neuman (1902) ...				almost—		
		Heubner (1903) ...				almost—		
		Hess and Fish (1914) ...				almost—		
		Miller (1917) ...				almost—		
		Cavanaugh, Dutcher, and Hall (1924)				— to +		
		Seligmann and co-workers (1926)				+		
		Miura (1929) ...				+		
		Schmidt-Nielsen (1929) ...					+	
		Hannemann (1928) ...					+	
	Pasteurised (Irradiated) Cows' : Whole Raw	Drummond (1918)...	++ to +++					
		Drummond, Coward, and Watson (1921)	+++					
		Chick and Roscoe (1926) ...	++ to +++					
		Dutcher, Honeywell, and Dahle (1927)	+++					
		Sherman (1926) ...	+++	++				
		Outhouse, Macy, Brekke, and Graham (1927)	+++	++	++			
		Hopkins (1906-12) ...		+to++				
		Osborne and Mendel (1911- 22)		+to++				
		Cooper (1912, 1914) ...		— to +				
		Gibson (1913) ...		— to +				
		Gibson and Concepcion(1916)		— to +				
		McCollum and Kennedy (1916)		— to +				
		Chick and Hume (1917) ...		Very low				
		Hughes, Fitch, and Cave (1921)		—to++				

Class of Foodstuffs.	Individual Foodstuff.	References.	VITAMIN					
			A	B ₁	B ₂	C	D	E
ANIMAL FOODSTUFFS —continued.	Milk— Cows': Whole Raw—contd.	Kennedy and Dutcher (1922)		— to +				
		Simonnet (1922) ...		— to +				
		Morgan and Francis (1924)		+ to ++				
		Bechdel, Honeywell, and co-workers (1927, 1928)		++				
		Daniels, Jordan, and Hutton (1929)		++				
		Sherman and Axtmayer (1927)		— to +	+			
		Hunt and Krauss (1928) ...		— to +	++			
		Goldberger and co-workers (1928)			++			
		Aykroyd (1928) ...			++			
		Chick, Hume, and Skelton (1918)				— to +		
		Barnes and Hume (1919)...				— to +		
		Dutcher <i>et al.</i> (1920) ...				+		
		Hess, Unger, and Supplee (1920)				— to +		
		Reyher (1926) ...				— to +		
		MacLeod (1927) ...				+		
		Hughes, Fitch, Cave, and Riddell (1927)				+		
		Mellanby (1919, 1921) ...					+ to ++	
		Paton and Watson (1921)					±	
		Hess and Unger (1921, 1922)					— to +	
		Chick, Dalyell, and MacKay (1923)					+ to ++	
		Korenchevsky (1923) ...					almost—	
		Leané and Vagliano (1924)					— to +	
		Luce (1924) ...					— to +	
		Boas and Chick (1924) ...					— to +	
		Kieferle and Zeiler (1926)					± to +	
		Hess and Weinstock (1927)					— to +	
		Outhouse, Macy, and Brekke (1928)					±	
		Burn (1930) ...					±	
		Honeywell and co-workers (1930)					±	
		Evans and Bishop (1923)...						± to +

Class of Foodstuffs.	Individual Foodstuff.	References.	VITAMIN					
			A	B ₁	B ₂	C	D	E
ANIMAL FOODSTUFFS —continued.	Milk— Cows': Whole Raw—contd.	Evans and Burr (1927) ...						± to +
		Titus, Hughes, Henshaw, and Fitch (1926)	—					
	Cows': Whole Raw (Irradiated)	Supplee and Dow (1927) ...	+++				+++	
		Drummond (1927)... ..	—					
		Nabarro and Hickman (1930)	+++				+++	
		Reyher (1926)				— to +		
		Cowell (1925)					++	
		Kramer (1925)					++	
		Steenbock, Hart, and Black (1925)					++	
		Daniels, Pyle, and Brooks (1926)					++	
		Hess and Weinstock (1927)					++	
		Degkwitz (1928)					++	
		Wieland (1928)					++	
		Scheer (1928)					++	
		Sobel and Claman (1929)					++	
		Coward (1929)					— to +	
		Watson and Findlay (1929)					++	
	Goats' ...	U.S. Dept. of Agric. Bur. Animal Industry (Animal Husbandry Div. (unpublished)	+++					
		Hunt and Winter (1922) ...				— to +		
		Meyer and Nassau (1924)				— to +		
		Steenbock, Hart, Hoppert, and Black (1925)					++	
	Human ...	Drummond, Coward, and Watson (1921)	+ to ++					
		Outhouse, Macy, Brekke, and Graham (1927)	++					
		Osborne and Mendel (1922)		+				
		Hoobler, Macy, and co- workers (1926)		— to +				
		Macy, Outhouse, Graham, and Long (1927)		+	— to +			
		Hess (1920)... ..				+		
		Leené and Vagliano (1924)					— to +	
		Kennedy and Palmer (1925)					+	

Class of Foodstuffs.	Individual Foodstuff.	References.	VITAMIN					
			A	B ₁	B ₂	C	D	E
ANIMAL FOODSTUFFS —continued.	Milk— Human— <i>contd.</i>	Meyer and Nassau (1925)				— to +		
		Macy and Outhouse (1928)				— to +		
		Hess and Weinstock (1927)					+	
		Hess, Weinstock, and Sherman (1927)					+ to ++	
		McCollum, Simmonds, Becker, and Shipley (1927)					+ to ++	
		Outhouse, Macy, and Brekke (1928)					+ to ++	
		Eufinger and co-workers (1929)					+ to ++	
		Guggisberg (1929) ...					+ to ++	
	Meat Juice ...	Sherman (1926) ...		+				
		Parsons (1920) ...		D		— to +		
		Chick and Hume (1917) ...				— to +		
	Pancreas ... (Sweetbreads)	Drummond (1918)... ..	+					
		Sherman (1926)	+	+				
		Eddy (1916, 1917)... ..		++				
	Pork	Wright (1923)	— to +					
		Hoagland and Snider (1925)	— to +					
		Sherman (1926)	— to +					
		Hoagland (1929)		++	++			
		Sherman (1926)				+		
		(Salt) Goldberger and co-workers (1930)			±			
	Poultry	Hoagland and Lee (1926)	— to +					
	Veal	Hoagland and Snider (1925)	— to +					
		Sherman (1926)		+				
ANIMAL OILS AND FATS.	Beef Fat ...	Osborne and Mendel (1915)	++					
		Steenbock, Boutwell, and Kent (1918)	++					
		Drummond and Coward (1920)	++					
		Steenbock, Sell, and Bull (1921)	++					
		Sherman (1926)	++	—				
		Mellanby (1919, 1920, 1921)					± to +	
		Evans and Burr (1927) ...						+
	Butter ...	McCollum and Davis (1914, 1915)	+++					

Class of Foodstuffs.	Individual Foodstuff.	References.	VITAMIN					
			A	B ₁	B ₂	C	D	E
ANIMAL OILS AND FATS —continued.	Butter —continued.	Osborne and Mendel (1915, 1921)	+++					
		McCollum, Simmonds, and Steenbock (1917)	+++					
		Drummond (1919)... ..	+++					
		Aron and Gralka (1921) ...	+++					
		Appleton (1921)	+to++					
		Drummond, Coward, and Watson (1921)	+to++					
		Steenbock, Boutwell, and Kent (1921)	+to++					
		Zilva and Miura (1921) ...	+++					
		Channon, Drummond, and Golding (1924)	+to++					
		Jones, Steenbock, and Nelson (1924)	++					
		Munsell (1924)	++					
		Rosenheim and Webster (1927)	+to++					
		Storms (1927)	+to++					
		Sherman (1926)		—				
		<i>Med. Res. Council Report</i> (1924-8)				—		
		Mellanby (1919, 1920, 1921)					± to +	
		McCollum, Simmonds, Becker and Shipley (1926)					± to +	
		Coward (1928)					± to +	
		Mattill and Clayton (1926)						± to +
		Sure (1926, 1927)						± to +
		Evans and Burr (1927) ...						Very low
		Tso (1927)						± to +
	Cream	Sherman (1926)	+++	++		— to +		
		<i>Med. Res. Council Report</i> (1924-8)	++	+(low)				
		Platon (1927)	++					
		Mellanby (1931)	++				±	
		Hess and Weinstock (1927)					±	
		Schultz and Maurmann (1928)					+	
	Ice Cream (Irradiated)	Russell, Button, and Kehlenberg (1929)						+

Class of Foodstuffs.	Individual Foodstuff.	References.	VITAMIN					
			A	B ₁	B ₂	C	D	E
ANIMAL OILS AND FATS —continued.	Fish (Fats)— Eel Salmon	Rosenheim and Webster(1927)	++					
		Bills (1927)					++	
		Matzko (1929)					+	
	Fish Liver Oils— Cod-Liver Oil	Drummond (1918, 1924, 1930)	+ to +++					
		Zilva, Giesser, and Miura (1921)	+ to +++					
		Zilva and Miura (1921) ...	+++					
		Drummond and Zilva (1922)	+++					
		Hjort (1922)	+ to +++					
		Holmes (1922, 1924) ...	+++					
		Javillier, Baude, and Levy-Lajeunesse (1924)	+ to +++					
		Jones, Steenbock, and Nelson (1924)	+++					
		Poulsso (1924, 1926, 1930)	+++					
		Zilva, Drummond, and Graham (1924)	+ to +++					
		Holmes and Piggott (1926)	+++					
		Munsell and Black (1928) ...	+++					
		Vedder (1912)		—				
		Lax (1921)		—				
		Sherman (1926)		—		—		
		Cohen and Mendel (1918)				—		
		Bezssonoff (1923)				—		
		Mellanby (1921)					+++	
		McCollum, Simmonds, Becker and Shipley (1922)					+ to +++	
		Gerstenberger and Nourse (1926)					+++	
		Grant and Goettsch (1926)					+++	
		Heuser and Norris (1926)...					+++	
		Adams and McCollum (1928)					+ to +++	
		Hess, Bills, and Honeywell (1928)					+ to +++	
		Lesné, Clement, and Simon (1928)					± to ++	
		Simmonds, Becker, and McCollum (1928)					+++	

Class of Foodstuffs.	Individual Foodstuff.	References.	VITAMIN					
			A	B ₁	B ₂	C	D	E
ANIMAL OILS AND FATS —(continued).	Fish Liver Oils— Cod-Liver Oil —continued.	Van Leersum (1928) ...					+++	
		Evans and Burr (1927) ...						— to +
		Sure (1927) ...						±
		Nelson and co-workers (1928)						— to +
	(Irradiated)	Wyman and co-workers (1926)					++	
		Daniels and Brooks (1927)					++	
		Van Leersum (1930) ...					± to +	
		Zilva and Miura (1921) ...	++					
	Other Fish Liver Oils, including Ling, Plaice, Pollock, Shark, Skate	Zilva and Drummond (1922)	++					
		Rosenheim and Webster (1927)	+++					
		Salmon ...	++				++	
		Burbot ...					+++	
	Salmon and Halibut	McCollum, Simmonds, Becker and Shipley (1912)					++	
		Puffer-fish ...					+++	
		Rat-fish ...	++					
		Fish Oils (Body) Cod, Coal-fish, and Haddock	++					
	Herring ...	Drummond (1921)... ...	++					
		Bills (1927)... ...					++	
		Salmon ...	+					
		Davis and Beach (1926, 1928)	++				++	
	Sardine ...	Schmidt-Nielsen (1929) ...	+					
		Bills (1927)... ...					++	
		Ghee ...	+					
		Thomas (1927) ...	almost—					
	Horse Fat ...	Bacharach (1930) ...	almost—					
		Coward and Drummond (1920)	+					
		Sherman (1926) ...		—		—		
		Lard ...	usually —					
	Lard ...	Osborne and Mendel (1915)	usually —					
		Drummond, Golding, Zilva, and Coward (1920)	usually —					

Class of Foodstuffs.	Individual Foodstuff.	References.	VITAMIN					
			A	B ₁	B ₂	C	D	E
ANIMAL OILS AND FATS —continued.	Lard—continued. (Irradiated)	Daniels and Loughlin (1920)	+					
		Mallon and Clark (1922) ...	— to +					
		Sherman (1926) ...	— to +	—		—		
		Goldberger and co-workers (1930)			±			
		Evans and Burr (1927) ...						—
		Steenbock and Black (1925)					—	
	Mutton Fat ...	Drummond and Coward (1920)	+ to ++					
		Sherman (1926) ...		—		—		
	Oleo-Margarine	Osborne and Mendel (1915)	+					
		Halliburton and Drummond (1917)	+					
		Williams and MacLennan (1929)	± to +					
		Sherman (1926) ...		—		—		
	Oleo Oil ...	Osborne and Mendel (1915)	+					
		Halliburton and Drummond (1917)	+					
		Hoagland and Snider (1926)	+ to ++					
		Sherman (1926) ...		—		—		
	Pig Fat ... (Perirenal) (Subcutaneous)	Drummond, Golding, Zilva, and Coward (1920)	++					
		Sherman (1926) ...		—		—		
		Drummond and Coward (1920)	±					
		Evans and Burr (1927) ...						+
	Poultry Fat ...	Hoagland and Lee (1926)	+					
	Pulmonary Fat	Roger, Binet, and Vagliano (1924)	+				+	
VEGETABLE OILS AND FATS.	Almond Oil ...	Osborne and Mendel (1914)	—					
		Morgan, Newbecker, and Bridge (1923-4)	—					
	Arachis or Peanut Oil (Irradiated)	Drummond and Coward (1920)	±					
		Stammers (1921) ...	—					
		Drummond and Zilva (1922)	+					
		Sherman (1926) ...		—		—		
		Evans and Burr (1927) ...						Low
		Sure (1926) ...						— to +
		Rondoni (1926) ...					+	

Class of Foodstuffs.	Individual Foodstuff.	References.	VITAMIN					
			A	B ₁	B ₂	C	D	E
VEGETABLE OILS AND FATS —continued.	Coco-nut Oil ...	Halliburton and Drummond (1917)	—					
		McCollum and co-workers (1921)	—					
		Ghose (1922)	— to +					
		Sherman (1926)		—		—		
		Mellanby (1921)					±	
		Evans and Burr (1927) ...						Low
	(Irradiated)	Steenbock and Black (1925)					+	
	Cocoa Butter ...	Halliburton and Drummond (1917)	—					
		McCollum, Simmonds, Becker and Shipley (1922)					+	
		Sure (1926)... ..						—
	(Irradiated)	Steenbock and Black (1925)					+	
	Corn Oil (Crude)	Drummond and Zilva (1922)	+					
		Meyer and Hetler (1929) ...	+					
		(Refined) ...						
		Steenbock and Coward (1927)	—					
		Meyer and Hetler (1929) ...	Low					
		Sherman (1926)		—		—		
		Musselhl, Hill, and Rosenbaum (1926)					— to +	
		Evans and Burr (1927) ...						Low
		Sure (1927)						— to +
		(Irradiated)						
		Steenbock and Black (1925)					+	
		Steenbock and Daniels (1925)					+	
		Musselhl, Hill, and Rosenbaum (1926)					+	
	Cotton-seed Oil	McCollum and co-workers (1917)	— to +					
		Halliburton and Drummond (1917)	— to +					
		Drummond and Coward (1920)	— to +					
		Sherman (1926)		—		—		
		Mellanby (1921)					±	
		McCollum, Simmonds, Becker, and Shipley (1922)					±	
		Sure (1926)... ..						± to +
		Evans and Burr (1927) ...						± to +

Class of Foodstuffs.	Individual Foodstuff.	References.	VITAMIN					
			A	B ₁	B ₂	C	D	E
VEGETABLE OILS AND FATS —continued.	Cotton-seed Oil —continued. (Irradiated) ... (Hydrogenated)	Hess and Weinstock (1924)					+	
		Drummond and Coward (1920)	—					
	Fat, Vegetable (Hydrogenated)	Mellanby (1921) ...					—	
		Kennedy and Palmer (1926)					—	
	Linseed Oil ...	McCollum and co-workers (1917)	±					
		Stammers (1921) ...	±					
		Drummond and Zilva (1922)	+					
		Evans and Burr (1927) ...						Low
	(Hydrogenated)	Drummond and Coward (1920)	—					
	(Irradiated) ...	Karóly (1927) ...					+	
	Maize Oil ...	Drummond and Coward (1920)	— to +					
		Stammers (1921) ...	+					
		Drummond and Zilva (1922)	+					
		Sherman (1926) ...		—		—		
		McCollum, Simmonds, Becker and Shipley (1922)					+	
		Evans and Burr (1927) ...						Low
		Sure (1926)...						— to +
	(Irradiated) ...	Steenbock and Black (1925)					+	
	Margarine (Vegetable)	Halliburton and Drummond (1917)	—					
	(Ghee) ...	Thomas (1927) ...	—					
		Sherman (1926) ...		—		—		
	Olive Oil ...	McCollum and Davis (1913)	—					
		Osborne and Mendel (1914)	—					
		Drummond and Coward (1920)	— to +					
		Javillier and Emérique (1929)	— to +					
		Sherman (1926) ...		—		—		
		McCollum, Simmonds, Becker, and Shipley (1922)					±	
		Sure (1926)...						— to +
	(Irradiated) ...	Steenbock and Black (1925)					+	
		Steenbock and Daniels (1925)					+	

Class of Foodstuffs.	Individual Foodstuff.	References.	VITAMIN					
			A	B ₁	B ₂	C	D	E
VEGETABLE OILS AND FATS —continued.	Olive Oil (Irradiated) —continued.	Brahm and Mendel (1926)					+	
		Karóly (1927)					+	
	Orange Peel Oil	Cooper (1921)	+					
	Palm Oil ...	Drummond and Coward (1920)	±					
		Drummond and Zilva (1922)	+ to + +					
	Sesame Oil ...	Drummond and Coward (1920)	—					
		Delf (1924)	—					
		McCollum, Simmonds, Becker, and Shipley (1922)					±	
	Soy Bean Oil ...	McCollum, Simmonds, and Pitz (1916-17)	—					
		Drummond and Zilva (1922)	+					
		Sure (1926)... ..						— to +
	Wheat Germ Oil	Sure (1925)... ..	±					
		Sure (1926)... ..						+++
		Evans and Burr (1927) ...						+++
		Daniels and Jordan (1928)						+++
CEREALS AND CEREAL PRODUCTS.	Adlay (<i>Coix lachryma</i>)	Santos and Collado (1925)		++				
	Barley (Unhusked) ...	Steenbock, Kent, and Gross (1918)	+	+				
		Southgate (1924)	±	++				
		Cooper (1912, 1914) ...		+				
		Osborne and Mendel (1920)		±				
		Harden and Zilva (1924) ...		+				
		Widmark (1924)		+				
		Plimmer and Rosedale (1927)		+				
		Cohen and Mendel (1918)				—		
	(Husked) ...	Cooper (1912, 1914) ...		±				
	(Sprouted) ...	Cohen and Mendel (1918)				++		
		McClendon and Cole (1919)				++		
		Schittenhelm and Eisler (1928)	+	++			+	Probable
	Bread (White)	Med. Res. Council Report (1924-8)	—					
		Oseki (1914)		— to +				

Class of Foodstuffs.	Individual Foodstuff.	References.	VITAMIN					
			A	B ₁	B ₂	C	D	E
CEREALS AND CEREAL PRODUCT —continued.	Bread (White) —continued.	Hara (1924)		— to +				
		Hartwell (1924)		+				
		Cramer and Mottram (1927)		Low				
		Sherman (1926)				—		
		Holst and Frölich (1912) ...				—		
		<i>Med. Res. Council Report</i> (1924–8)	+			—		
		Sherman (1926)	+					
		Cramer and Mottram (1927)		++				
	Corn (Maize) ...	Steenbock and Coward (1927)	— to +					
	(Embryo) ...	Chick and Hume (1917, 1927)		++				
		Croll and Mendel (1925) ...		++				
		Jansen and Donath (1928)		++				
		Hauge and Carrick (1926)			Low			
		Aykroyd and Roscoe (1929)			Very low			
		Evans and Burr (1927) ...						+
		(Endosperm)						
		Steenbock and Coward (1927)	+					
		Hauge and Trost (1928) ...	+					
		Rocke, Plant, and Hetler (1928)	+					
		Russell (1930)	+					
		Croll and Mendel (1925) ...		±				
		Jansen and Donath (1928)		— to ±				
		Aykroyd and Roscoe (1929)			almost—			
	Gluten ...	Johns, Finks, and Paul (1920)	+					
		Meyer and Hetler (1929) ...	+					
	Corn (Maize): Whole (White)	McCollum, Simmonds, and Pitz (1916)	—					
		Steenbock and Boutwell (1920)	—					
		Coward and Drummond (1921)	—					
		Hauge and Trost (1928) ...	—					
	Corn (Maize): Whole (Yellow)	McCollum, Simmonds, and Pitz (1916)	+					
		Steenbock and Boutwell (1920)	+					

Class of Foodstuffs.	Individual Foodstuff.	References.	VITAMIN					
			A	B ₁	B ₂	C	D	E
CEREALS AND CEREAL PRODUCTS —continued.	Corn (Maize): Whole (Yellow) —continued.	Coward and Drummond (1921)	±					
		Steenbock and Coward (1927)	+					
		Russell (1930) ...	+					
		Hogan (1916) ...		+				
		McCollum, Simmonds, and Pitz (1916)		+				
		Chick and Hume (1917) ...		+				
		Hughes (1918) ...		+				
		Voegtlin and Myers (1918)		+				
		Jansen and Donath (1928)		± to +				
		Hunt (1928) ...			±			
		Aykroyd and Roscoe (1929)			±			
		Sherman (1926) ...				—		
		Steenbock, Black, and Thomas (1927)					Low	
	(Irradiated)...	Steenbock and Daniels (1925)					+	
		Mussell, Hill, and Rosenbaum (1926)					±	
	(Young Cobs)	<i>Med. Res. Council Report</i> (1924-8)				±		
	Corn, Kaffir (<i>Sorghum vulgare</i>)	<i>Med. Res. Council Report</i> (1924-8)		++				
	(Germinated)	Delf (1921) ...				+		
	Corn Starch ...	Evans and Burr (1928) ...			+			
	(Irradiated)...	Steenbock and Daniels (1925)					+	
	Custard Powders and Egg Substitutes	<i>Med. Res. Council Report</i> (1924-8)	—					
	Flour (White)	Sheehy (1927) ...	almost—					
		Chick and Hume (1917) ...		—				
		Osborne and Mendel (1919)		—				
		Voegtlin, Sullivan, and Myers (1919)		—				
		Bell and Mendel (1923) ...		—				
		Randoin and Simonnet (1927)		almost—				
		Cramer and Mottram (1927)		—				

Class of Foodstuffs.	Individual Foodstuff.	References.	VITAMIN					
			A	B ₁	B ₂	C	D	E
CEREALS AND CEREAL PRODUCTS —continued.	Flour (White) —continued.	Hartwell and Mottram (1929)			almost—			
		McCollum and Simmonds (1928)			—			
		Holst and Frölich (1912)				—		
		Cohen and Mendel (1918)				—		
		Mellanby (1922) ...					—	
	(Irradiated) ...	Hess and Weinstock (1925)					+	
		Steenbock and Daniels (1925)					+	
	(Wholemeal or Brown)	Sheehy (1927) ...	±					
		Randoin and Simonnet (1927)		±				
		Cramer and Mottram (1927)		±				
	(Wholemeal)...	Hartwell and Mottram (1929)			±			
		Cohen and Mendel (1918)				—		
	Malt ...	<i>Med. Res. Council Report</i> (1924-8)	—					
		Southgate (1924) ...	±					
		Sherman (1926) ...	+					
		Cooper (1912) ...		—				
		Harden and Zilva (1924) ...		+				
		Randoin and Lecoq (1927)		+				
		Bacharach and Allchorn (1928)		+				
		Fürst (1912) ...				—		
		McClendon, Cole, Engstrom, and Middlekauf (1919)				+		
		Randoin and Lecoq (1927)				+		
	(Kilned) ...	<i>Med. Res. Council Report</i> (1924-8)		— to ++				
		Harden and Zilva (1918) ...				—		
	Millet ...	McCollum, Simmonds, and Pitz (1917)	±					
		Steenbock, Sell, and Jones (1923)	± to +					
		<i>Med. Res. Council Report</i> (1924-8)	++					
		Auer (1919) ...	±					
		McCollum (1917) ...		+				
	(Chaomi) ...	Abe (1928) ...		+				

Class of Foodstuffs.	Individual Foodstuff.	References.	VITAMIN					
			A	B ₁	B ₂	C	D	E
CEREALS AND CEREAL PRODUCTS —continued.	Oats	McCollum, Simmonds, and Pitz (1917)	±					
		Steenbock and Coward (1920, 1927)	— to +					
		Bezssonoff (1929)	—					
		Osborne and Mendel (1920)		+				
		Plimmer and Rosedale (1927)		±				
		Rocke and Hetler (1928) ...		++				
		Cohen and Mendel (1918)...				—		
		Steenbock, Black, and Thomas (1927)					—	
	(Irradiated) ...	Bezssonoff (1929)	—					
		Steenbock and Daniels (1925)					+	
	(Sprouted) ...	Cohen and Mendel (1918)				+		
		Kucera (1928)				+		
	Oatmeal ...	Sherman (1926)	— to +	++		—		
		Plimmer and Rosedale (1927)		Low				
	Rice (Endosperm)	Edie and Simpson (1911)...		±				
		Chick and Hume (1917) ...		±				
	(Germ) ...	Eijkman (1897, <i>seq.</i>) ...		++				
		Holst and Frölich (1912) ...		++				
		Chick and Hume (1917) ...		++				
		Kintaro (1927)		++				
		Guerrero and Concepcion (1920)	+					
	(Polishings) ...	Jansen and Donath (1924)	+					
		Santos and Collado (1925)		+				
		Munsell (1930)		+	+			
		Evans and Burr (1929) ...			(?) +			
		Guerrero and Concepcion (1920)	+					
	(Whole) ...	McCarrison (1927)...		+				
		<i>Illinois Agric. Exp. Stat. Annual Report</i> (1927)		+				
		Kondo and co-workers (1929)		+				
		Sherman (1926)				—		
		Cook and Rivera (1929) ...					almost—	

Class of Foodstuffs.	Individual Foodstuff.	References.	VITAMIN					
			A	B ₁	B ₂	C	D	E
CEREALS AND CEREAL PRODUCTS—continued.	Rice—continued. (Polished) ...	McCollum and Davis (1915)	—					
		Guerrero and Concepcion (1920)	—	—				
		Jansen and Donath (1924)	—					
		Weill and Mouriquand (1914, 1916)		—				
		Chamberlain (1915) ...		—				
		Eijkmann (1916, etc.) ...		—				
		Chick and Hume (1917) ...		—		—		
		Chamberlain and Vedder (1918)		—				
		Illinois Agric. Exp. Stat. Annual Report (1927)		—				
		Sherman and Axtmayer (1930)		—	—			
		Mathur (1930) ...		—				
	Rye (Germ) ... (Whole) ...	Scheunert (1927) ...	+	++		—		
		Sherman (1926) ...	+			—		
		Osborne and Mendel (1920)		+				
		Guest, Nelson, Parks, and Fulmer (1926)		+				
		Plimmer and Rosedale (1927)		±				
	(Sprouted)	Kucera (1928) ...				+		
	Wheat (Germ)	McCollum, Simmonds, and Pitz (1916)	++					
		Scheunert (1927) ...	+					
		Steenbock and Coward (1927)	+					
		McCollum and co-workers (1915, 1916)		++				
		Chick and Hume (1917, 1919)		++				
		Daniels, Byfield, and Loughlin (1919)		++				
		Osborne and Mendel (1919)		++				
		Bell and Mendel (1922) ...		++				
		Cramer and Mottram (1927)		++				
		Morgan and Barry (1930)		++				
		Voegtlin and Myers (1920)			Low			
		Chick and Roscoe (1927) ...			+			

Class of Foodstuffs.	Individual Foodstuff.	References.	VITAMIN					
			A	B ₁	B ₂	C	D	E
CEREALS AND CEREAL PRODUCTS —continued.	Wheat (Germ) —continued.	Goldberger and Wheeler (1927)			+			
		Aykroyd and Roscoe (1929)			+			
		Morgan and Barry (1930)			++			
		Holst and Frölich (1912) ...				—		
		Hess (1916)				—		
		Evans and Bishop (1923)...						++
		Evans and Burr (1927) ...						++
	(Bran)	Stammers (1921)	+					
		Chick and Hume (1917) ...		— to +				
		Bell and Mendel (1922) ...		±				
		Cramer and Mottram (1927)		±				
		Aykroyd and Roscoe (1929)			+			
		McCollum, Simmonds, and Pitz (1916)	—					
		Chick and Hume (1917) ...		+				
	(Endosperm)	Osborne and Mendel (1919)		+				
		Stammers (1921)		+				
		Bell and Mendel (1922) ...		++				
		Aykroyd and Roscoe (1929)			Very low			
		Hart and McCollum (1914)	±					
		McCollum, Simmonds, and Pitz (1916)	±					
		Steenbock and Coward (1927)	+					
	(Whole) ...	McCollum, Simmonds, and Pitz (1916)		++				
		Osborne and Mendel (1919)		++				
		Bell and Mendel (1922) ...		++				
		McCarrison (1927)		++				
		Hunt (1927)		++				
		Goldberger and Wheeler (1927)			±			
		Sherman and Axtmayer (1927)			±			
		Hunt (1928)			±			
		Aykroyd and Roscoe (1929)			±			
		Holst and Frölich (1912) ...				—		
		Evans and Burr (1927) ...						+
		Steenbock and Daniels (1925)					+	
	(Irradiated)							

Class of Foodstuffs.	Individual Foodstuff.	References.	VITAMIN					
			A	B ₁	B ₂	C	D	E
SEEDS AND NUTS.	Almonds ...	Coward and Drummond (1920)	+					
		Swatz and McLeod (1922)	++					
		Morgan, Newbecker, and Bridge (1923-4)	++					
		Cajori (1920) ...		++				
		Plimmer and co-workers (1929)		+				
	(Sprouted) ...	Fürst (1912) ...				+		
	Arachis Seed ...	Drummond and Zilva (1922)	Very low					
		<i>Med. Res. Council Report</i> (1924-8)				Probably absent		
	Babassu Seed...	Drummond and Zilva (1922)	Very low					
	Barcelona Nuts	Coward and Drummond (1920)	Very low					
		<i>Med. Res. Council Report</i> (1924-8)		++		Probably absent		
	Beechnuts ...	Sherman (1926) ...		++				
	Brazil Nuts ...	Coward and Drummond (1920)	Very low					
		Cajori (1920) ...		++				
		<i>Med. Res. Council Report</i> (1924-8)		++		Probably absent		
	Butternuts ...	Coward and Drummond (1920)	Very low					
		Sherman (1926) ...		++				
	Cabbage Seed...	<i>Med. Res. Council Report</i> (1924-8)	Very low					
	Cacao ...	Drummond and Zilva (1922)	D					
	Candlenuts ...	Drummond and Zilva (1920)	Low					
	Carrot Seed ...	Coward and Drummond (1920)	+					
	Chestnuts ...	Cajori (1920) ...		+				
		Plimmer and co-workers (1929)		+				
	Citician Seed ...	Drummond and Zilva (1922)	—					
	Coconut ...	Sherman (1926) ...	+	++				
		<i>Med. Res. Council Report</i> (1924-8)		++				
		Plimmer and co-workers (1929)		—				
	(Press Cake)...	Johns, Funks, and Paul (1919)	+	++				

Class of Foodstuffs.	Individual Foodstuff.	References.	VITAMIN					
			A	B ₁	B ₂	C	D	E
SEEDS AND NUTS —continued.	Coconut (Press Cake) —continued.	Jansen and Donath (1924)	+					
		Evans and Burr (1927) ...						+
	Coffee Beans ...	<i>Med. Res. Council Report</i> (1924-8)		+				
	(Green) ...	Plimmer and co-workers (1929)		+				
	(Roasted) ...	Mattei (1923) ...		±				
		Plimmer and co-workers (1929)		—				
	Cohune Seed ...	Drummond and Zilva (1922)	—					
	Copra ...	Drummond and Zilva (1922)	—					
	Cotton Seed ... (Meal) ...	Osborne and Mendel (1917)	+					
		Drummond and Zilva (1922)	D					
		McCollum (1917) ...		+				
		Richardson and Green (1916, 1917)	+	+				
		McCollum (1917) ...		+				
		Greig and Curjel (1919) ...		+				
	Djave Seed ...	Drummond and Zilva (1922)	—					
	Fennel Seed ...	Drummond and Zilva (1922)	—					
	Filberts ...	Cajori (1920) ...		++				
		<i>Med. Res. Council Report</i> (1924-8)		++				
	Hazel Nuts ...	Plimmer and co-workers (1929)		+				
	Hickory Nuts...	Cajori (1920) ...		++				
	Kapok Seed ...	Drummond and Zilva (1922)	D					
	Linseed ... (Cake) ...	McCollum, Simmonds, and Pitz (1917)	++					
		Drummond and Zilva (1922)	++					
		McCollum (1917) ...	++	++				
		Drummond and Zilva (1922)	+					
	Litchi Nuts ...	Smith and Sah (1927) ...	—	—				
	Millet Seed ...	McCollum, Simmonds, and Pitz (1917)	++					
		Auer (1919) ...	±					
		Steenbock, Sell, and Jones (1923)	+ to ++					
		McCollum (1917) ...	++	++				

Class of Foodstuffs.	Individual Foodstuff.	References.	VITAMIN					
			A	B ₁	B ₂	C	D	E
SEEDS AND NUTS —continued.	Palm Kernels...	Drummond and Zilva (1922)	+					
	Peanuts ...	Coward and Drummond (1920)	Very low					
		Daniels and Laughlin (1918)	Low	++				
		Osborne and Mendel (1917)		++				
		Greig (1919) ...		++				
		Johns and Finks (1920) ...		++				
		Evans and Burr (1927) ...						+
	Pecans... ..	Salmon and Livingstone (1925)	+	++				
		<i>Med. Res. Council Report</i> (1924-8)		++				
	Pine Nuts ...	Sherman (1926)	+	+				
		<i>Med. Res. Council Report</i> (1924-8)		++				
	Rape Seed ...	Drummond and Zilva (1922)	Low					
	Sesame Seed ...	Drummond and Zilva (1922)	Low					
	Sunflower Seed	<i>Med. Res. Council Report</i> (1924-5)	D					
	Walnuts (Black) (English)	<i>Med. Res. Council Report</i> (1924-8)		++				
		Coward and Drummond (1920)	Very low	+++				
		Mignon (1923)	+					
		Cajori (1920)		++				
VEGETABLES AND VEGETABLE PRODUCTS.	Alfalfa (Lucerne)	McCollum, Simmonds, and Pitz (1916)	++					
		Osborne and Mendel (1919)	++	+				
		Steenbock and Boutwell (1920)	+					
		Steenbock and Gross (1920)	++	+				
		Palmer and Kennedy (1921)	+					
		McCollum, Simmonds, Becker and Shipley (1922)	++					
		McCollum (1917)		+				
		Osborne and Mendel (1920)		+				
		Steenbock and Nelson (1923)					Almost nil	
		Evans and Burr (1927) ...						+
	Artichokes ...	Morgan and Stephenson (1923)	+	+				
		Santos (1922)		+				

Class of Foodstuffs.	Individual Foodstuff.	References.	VITAMIN					
			A	B ₁	B ₂	C	D	E
VEGETABLES AND VEGETABLE PRODUCTS— continued.	Asparagus ...	Crist and Dye (1929) ...	+++					
		Osborne and Mendel (1922)		++				
	Bamboo Shoots	Santos (1922) ...		+				
	Beans (Katjangidjo)	Grijns (1901) ...		++				
		Pol (1917) ...		++				
	Kidney ...	Remy (1928) ...				—		
	(Canned) ...	Med. Res. Council Report (1924-8)	Low (+)					
	(Raw) ...	Sherman (1926) ...	+	+				
	(Red) ...	Shimoda, Fujimaki, and Saiki (1927)		+				
		Axtmayer (1930) ...		+	+			
	Mung... ...	Embrey (1921) ...	+					
		Heller (1927) ...	+					
		Santos (1922, 1921) ...		++		Low		
		Cohen and Mendel (1918)				Low		
	(Sprouted)	Miller and Hair (1928) ...	+	+		++		
		Simonik (1929) ...				++		
	Navy (Canned)	Sherman (1926) ...	+	+				
	(Raw) ...	McCollum, Simmonds, and Pitz (1917)		+				
	Rangoon ...	Med. Res. Council Report (1924-8)	—					
	Soy ...	Osborne and Mendel (1917)	Low (+)	++				
		Jansen and Donath (1924)	+					
		Sherman (1929) ...		++				
		Salmon (1927) ...		+	+			
		Cohen and Mendel (1918)				—		
	(Sprouted)	Delf (1921) ...				D		
	String (Canned)	Fong (1927) ...		+				
		Campbell and Chick (1919)				D		
	(Raw)	Munsell (1924) ...	+					
		Sherman (1926) ...	+					
		Quinn, Burtis, and Milner (1927)	++	++		+		
		Campbell and Chick (1919)				+		
	Velvet (Georgia)	Salmon, Guerrant, and Hays (1928)		+	+			

Class of Foodstuffs.	Individual Foodstuff.	References.	VITAMIN					
			A	B ₁	B ₂	C	D	E
VEGETABLES AND VEGETABLE PRODUCTS—continued.	Beans Velvet (Georgia)—continued.	Salmon (1927)			+			
		Sure (1926)						+
	Beet	Steenbock and Gross (1920)	±					
		Osborne and Mendel (1920)		++				
		Scheunert (1927)		++		+		
		Chick and Dalyell (1919) ...				+		
		(Juice)				+		
		Russell and Morrison (1922)				+		
		Med. Res. Council Report (1924-8)	Low			+		
		Chick and Rhodes (1918)...				+		
		(Leaves and Stem)						
		Sherman (1926)	++					
		Osborne and Mendel (1919)		++				
	Cabbage (Green Leaves)	Hume (1921)	++					
		Coward and Drummond (1921)	++					
		Steenbock and Sell (1922)	++					
		Osborne and Mendel (1919, 1920)	+	++				
		McCollum and Kennedy (1916)		++				
		Chick and Hume (1917) ...		++		++++		
		Dunham (1921)		++				
		Roscoe (1930)			+			
		Delf and Tozer (1918) ...				+++		
		Delf and Skelton (1918) ...				+++		
		Chick and Dalyell (1919)...				+++	+	
		Mellanby and Killick (1926)						+
		Sure (1926)						
	(White Leaves)	Coward and Drummond (1921)	—					
		Hume (1921)	—					
		Steenbock and Sell (1922)	— to +					
		Med. Res. Council Report (1924-8)				+++		
		(Chinese)				++		
		(Canned)				+		
		Campbell and Chick (1919)				+		

Class of Foodstuffs.	Individual Foodstuff.	References.	VITAMIN					
			A	B ₁	B ₂	C	D	E
VEGETABLES AND VEGETABLE PRODUCTS— continued.	Cabbage (Dried) ...	Osborne and Mendel (1919)	++					
		Steenbock and Gross (1920)	++					
		<i>Med. Res. Council Report</i> (1924-8)		++				
		Shorten and Roy (1919, 1921)		++		+++		
		Givens and Cohen (1918)...				+++		
		Delf and Skelton (1918) ...				++		
		Holst and Fleischer (1927)				++		
		Rohmer (1929) ...				++		
	(Pickled) ...	Chick and Hume (1917) ...				—		
	Carrots (Canned)	Remy (1928) ...				—		
		(Dried)						
		Steenbock and Gross (1919)	+					
		<i>Med. Res. Council Report</i> (1924-8)		++				
		Hess and Unger (1919)		±		
		Shorten and Roy (1921) ...				—		
	Raw (Young)	Denton and Kohman (1918)	++	++				
		Steenbock and Gross (1919)	++	++				
		Osborne and Mendel (1920)	+++	++				
		Steenbock and Boutwell (1920)	++	++				
		Sherman (1926) ...	+++	++				
		Dunham (1921) ...		++				
		Shorten and Roy (1921) ...		++				
		Goldberger and Wheeler (1927)			+			
		Holst and Frölich (1912)...				++		
		Chick and Dalyell (1919) ...				++		
		Hess and Unger (1919) ...				++		
		Mellanby and Killick (1926)					±	
		Sherman and Hessler (1927)					±	
	(Old) ...	Munsell (1924) ...	+					
		Hess and Unger (1919) ...				+		
	White (Belgian)	Steenbock and Sell (1922)	Very low					
		Davis and Beach (1926) ...	Low					
	Juice ...	Chick and Rhodes (1918)...				+		

Class of Foodstuffs.	Individual Foodstuff.	References.	VITAMIN					
			A	B ₁	B ₂	C	D	E
VEGETABLES AND VEGETABLE PRODUCTS— continued.	Cauliflower (Canned) (Cooked) ... (Raw) ...	Remy (1928)			—		
		Russell, Morrison, and Ebling (1926)		±				
		Holst and Frölich (1912) ...				+		
		Sherman (1926)	+					
		Russell, Morrison, and Ebling (1926)		+				
		Holst and Frölich (1912) ...				+		
	Celery ... (Ethylene blanched)	Sherman (1926)	+					
		Osborne and Mendel (1922)		++				
		Babb (1928)		++				
	Chard	Steenbock and Gross (1920)	++					
		Sherman (1926)		++				
	Chayotes ...	Jansen and Donath (1924)	++					
	Clover	McCollum (1917)	++	+				
		Osborne and Mendel (1919, 1920)	++	+				
		Steenbock and Gross (1920)	++	+				
		Steenbock, Hart, and co-workers (1925)					+	
	Collards ...	Burton (1928)	+++	++		++		
		Georgia Agri. Expt. Stat. Amer. Report (1927)		++				
	Cow-peas ... (Sprouted)	Jansen and Donath (1924)	++					
		Acuna (1923)		++				
		Goldberger and Wheeler (1927)			±			
		Delf (1918, 1921)				+++		
	Cucumber ...	Jansen and Donath (1924)	— to +					
		Sherman (1926)		+				
		Embrey (1923)				++		
	Dandelion Greens	Sherman (1926)	++					
		Osborne and Mendel (1922)		+				
		Med. Res. Council Report (1924-8)		+				
		Holst and Frölich (1912) ...				+		
	Dasheen (Taro)	Steenbock and Gross (1919)	±	+				
		Miller (1927)		+		+		

Class of Foodstuffs.	Individual Foodstuff.	References.	VITAMIN					
			A	B ₁	B ₂	C	D	E
VEGETABLES AND VEGETABLE PRODUCTS— <i>continued.</i>	Egg Plant ...	Sherman (1926) ...	+	+				
		<i>Med. Res. Council Report</i> (1924-8)		+		+		
		(Dried) ... Shorten and Roy (1921) ...				+		
	Endive ...	Sherman (1926) ...	+					
		Holst and Frölich (1912) ...				+		
	Escarole ...	Sherman (1926) ...	+++					
	Kale ...	Sherman (1926) ...	++					
	Kohl-rabi ...	Holst and Frölich (1912)				+ (Low)		
	Lentils (Dried)	Jones and Murphy (1924)	+	++				
		Cooper (1912, 1914) ...		++				
		McCollum, Simmonds, and Pitz (1917)		++				
		Abderhalden and Schau- mann (1918)		+				
		Osborne and Mendel (1919)		++				
		Ghose (1922) ...		+				
		Plimmer and co-workers (1929)		+				
		Cohen and Mendel (1918)...				Low		
		Santos (1921) ...				Low		
		(Sprouted) Fürst (1912) ...				++		
		Chick and Delf (1919) ...				++		
	Lettuce ...	Steenbock and Gross (1920)	++					
		Munsell (1924) ...	++					
		Dye, Medlock, and Crist (1927)	++					
		Kramer, Boehm, and Wil- liams (1929)	++					
		Osborne and Mendel (1922)		+				
		Roscoe (1930) ...			+			
		Holst and Frölich (1912) ...				++		
		Hess and Weinstock (1924)					—	
		Evans and Burr (1927) ...						+++
		(Irradiated) Hess and Weinstock (1924, 1925)					+	
	Limu ...	Miller (1927) ...	+	+		—		
	Mangold ...	Steenbock and Gross (1919)	Very low					
		Davis and Beach (1926) ...	Very low					

Class of Foodstuffs.	Individual Foodstuff.	References.	VITAMIN					
			A	B ₁	B ₂	C	D	E
VEGETABLES AND VEGETABLE PRODUCTS— continued.	Mangold —continued.	<i>Med. Res. Council Report</i> (1924-8)		—				
		Boock and Trevan (1922)				+		
	Mushrooms ...	Coward and Drummond (1921)	Very low					
		Orton, McCollum, and Sim- monds (1922)		++				
		Hara (1923) ...		++				
		Mattei (1923) ...		++				
		Steidle (1924) ...				—		
		Sumi (1928) ...					±	
		Mellanby and co-workers (1929)					—	
	Nectar... ..	<i>Med. Res. Council Report</i> (1924-8)		—				
	Okra	Santos (1922) ...		+++				
	Onions (Raw)...	Hume (1921) ...	—					
		McCarrison (1919) ...		±				
		Osborne and Mendel (1919)		+				
		Orton, McCollum, and Sim- monds (1922)		+				
		Chick and Hume (1917) ...				++		
		(Dried) Shorten and Roy (1919, 1921)		+		+		
	Parsley ...	Osborne and Mendel (1922)		++				
	Parsnips ...	Steenbock and Gross (1919)	— to +					
		Sherman (1926) ...		++				
	Peas (Canadian Field) (Dried) ...	Evans and Burr (1927) ...						+
		McCollum, Simmonds, and Parsons (1919)	+	+				
		Cooper (1912, 1914) ...		+				
		Chick and Hume (1917) ...		±				
		McCollum, Simmonds, and Pitz (1917)		+				
		Osborne and Mendel (1919)		+				
		Plimmer and co-workers (1929)		+				
		McCollum and Simmonds (1918)			—			
		Aykroyd and Roscoe (1929)			±			
		Goldberger and co-workers (1930)			±			

Class of Foodstuffs.	Individual Foodstuff.	References.	VITAMIN					
			A	B ₁	B ₂	C	D	E
VEGETABLES AND VEGETABLE PRODUCTS— continued.	Peas (Dried) —(continued).	Chick and Delf (1919) ...				±		
	(Canned) ...	Eddy, Kohman, and Carlsson (1926)	++	+to++				
		Dye and Hershey (1928) ...	++	+to++				
		Eddy (1926)				++		
		Remy (1928)				++		
	(Green) ...	Steenbock, Sell, and Boutwell (1921)	++					
		Sherman (1926)	++	++				
		Eddy, Kohman, and Carlsson (1926)	++	++		+++		
		Fürst (1912)				+++		
		Chick and Delf (1919) ...				+++		
		Stevenson (1920)				+++		
		Simonik (1929)				+++		
	Peppers, Green (<i>Capsicum frutescens</i>)	Quinn, Burtis, and Milner (1927)	++	++		+++		
		Hermano (1930)	++	++				
	Poi (preparations of Taro, Dasheen)	Miller (1927)	+	+		+		
	Pollen	Med. Res. Council Report (1924-8)		++				
	Potatoes ...	McCollum, Simmonds, and Parsons (1918)	±	+				
		Steenbock and Gross (1919)	±	+				
		Osborne and Mendel (1920)	±	+				
		Steenbock and Sell (1922)	- to ±					
		Chick and Hume (1917) ...		±		++		
		Sherman (1926, 1927) ...		±		++		
		Lyman and Blystone (1926)		+				
		Holst and Frölich (1912)...				++		
		Chick and Rhodes (1918)...				++		
		Givens and McClugage (1919, 1920)				+		
	Pumpkin ...	Morgan and Francis (1924)	++	+				
		Jansen and Donath (1924)	++					
		Delf (1921)				+		
		Wats (1929)				++		
	Radishes ...	Morgan (1924)	-	++				

Class of Foodstuffs.	Individual Foodstuff.	References.	VITAMIN					
			A	B ₁	B ₂	C	D	E
VEGETABLES AND VEGETABLE PRODUCTS— continued.	Radishes —continued.	<i>Med. Res. Council Report</i> (1924-8)		+				
		Shimoda, Fujimaki, and Saiki (1927)				++		
	Rhubarb ...	Pierson and Dutcher (1920)				+		
	Romaine ...	Sherman (1926) ...	++	++				
	Sauerkraut ...	Sherman (1926) ...	+	+				
		Clow and co-workers (1929)				+ to ++		
		Clow and co-workers (1930)	Very low			Variable		
	Sorrel ...	Holst and Frölich (1912)				+		
	Spinach (Canned)	Eddy, Kohman, and Carls- son (1925)	++	+		+ to ++		
		Williams (1927) ...		+				
	(Dried) ...	Pierson, E. (1926) ...				+ to +++		
		<i>South Dakota Expt. Stat.</i> <i>Amer. Report</i> (1926-7)				+ to +++		
		McClendon and Schuck (1923)	++					
		Willimott and Wokes (1927)	++					
		Osborne and Mendel (1919)		+				
		Shorten and Roy (1919) ...		+		—		
		Steenbock and Gross (1920)		++				
		Eddy, Kohman, and Carls- son (1925)		+				
	(Raw) ...	Munsell (1924) ...	++					
		Eddy, Kohman, and Carls- son (1925)	+++		++	+++		
		Sherman (1926) ...	+++	++				
		Osborne and Mendel (1919, 1920)	+++	++				
		Steenbock and Gross (1920)	+++	++				
		Eddy (1927) ...			++			
		Roscoe (1927) ...			+			
		Shorten and Roy (1921) ...				++		
		Hess and Unger (1918) ...					—	
		Goldblatt and Zilva (1923)					— to ±	
		Zucker and Barnett (1923)					— to ±	
		Chick and Roscoe (1926) ...					— to ±	
		Boas (1926) ...					— to ±	
		Roscoe (1927) ...					±	

Class of Foodstuffs.	Individual Foodstuff.	References.	VITAMIN					
			A	B ₁	B ₂	C	D	E
VEGETABLES AND VEGETABLE PRODUCTS— <i>continued.</i>	Spinach (Irradiated)	Hess (1925)					+	
		Mackay and Shaw (1925)...					+	
		Chick and Roscoe (1926) ...					+	
	Squash, Hubbard	Steenbock and Boutwell (1920)	++					
	Sunflower Shoots	<i>Med. Res. Council Report</i> (1924-8)	++					
	Swedes (Ruta- baga)	Scheunert (1927)	++	+				
		Steenbock and Gross (1919)		+				
		Goldberger and Wheeler (1927)			- to +			
		Chick and Rhodes (1918)...				+++		
		Delf (1920, 1925)				+++		
		Mellanby and Killick (1926)					+	
	Sweet Potato	Steenbock and Gross (1919)	++	+				
		Sherman (1926)	++					
		Jansen and Donath (1928)	++					
		Santos (1921)		+				
		Delf (1921)				++		
		(Leaves and Shoots) Santos and Collado (1928)		++				
	Tannia... ..	Clark, A. (1929)		+				
		Browning, E. (1930) ...		+				
	Timothy Hay...	Osborne and Mendel (1919)	++					
		<i>Med. Res. Council Report</i> (1924-8)		++				
	Tomatoes (Canned)	Osborne and Mendel (1920)	++					
		Sherman and Munsell (1925)	++					
		Goldberger and Wheeler (1927)			±			
		Sherman, La Mer, and Campbell (1922)				++		
		Delf (1924)				+		
		Kohman and co-workers (1926)				++		
		Clow and Marlatt (1930) ...				+ to ++		
		(Raw) ... Osborne and Mendel (1920)	++	++				
	(Raw) ...	Sherman and Munsell (1925)	++					
		Morgan and Smith (1928)	++					

Class of Foodstuffs.	Individual Foodstuff.	References.	VITAMIN					
			A	B ₁	B ₂	C	D	E
VEGETABLES AND VEGETABLE PRODUCTS— <i>continued.</i>	Tomatoes (Raw) — <i>continued.</i>	Jones and Nelson (1930) ...	++					
		Eddy (1921) ...		++				
		Hartwell (1922) ...		++				
		Sherman and Gross (1923)		++				
		Sherman (1926) ...		++				
		Givens and McClugage (1919)				+++		
		Delf (1921) ...				+++		
		Sherman, La Mer, and Campbell (1922)				+++		
		Jones and Nelson (1930) ...				+++		
	(Artificially Ripened)	Morgan and Smith (1928)	++					
		House, Nelson, and Haber (1929)	++	++		+++		
		Jones and Nelson (1930) ...	+			+++		
		Nelson and Breese (1930)		+				
	Turnips ...	Sherman (1926) ...	— to +					
		Davis and Beach (1926) ...	— to +					
		Osborne and Mendel (1920)		+				
		Horvath (1926) ...		+				
		Goldberger and Wheeler (1927)			— to +			
		Shorten and Roy (1921) ...				+		
		Tso (1928) ...				+		
	(Greens) ...	Newton (1928) ...	+++	++				
		Burton (1928) ...	+++	++		++		
		Shorten and Roy (1919, 1921)				+		
		Scheunert (1927) ...				+		
	Vegetable Marrow	Delf (1921) ...				+		
	Watercress ...	Coward and Eggleton (1928)	+++			+++		
	Yautia (White) (Yellow)	Cook (1927) ...	+	+				
		Cook and Rivera (1929) ...	++	++		±		
FRUITS.	Apples (Canned)	Kohman, Eddy, and Carlsson (1924)				+ to ++		
	(Dried)	Givens, McClugage, and van Horne (1922)				Low		
	(Raw)...	Sherman (1926) ...	+	+		++		
		Munsell (1924) ...	±					

Class of Foodstuffs.	Individual Foodstuff.	References.	VITAMIN					
			A	B ₁	B ₂	C	D	E
FRUITS— <i>continued.</i>	Apples (Raw) — <i>continued.</i>	Osborne and Mendel (1919, 1920)		+				
		Eddy (1921) ...		+				
		Hartwell (1922) ...		+				
		Givens, McClugage, and van Horne (1912)				+		
		Kohman, Eddy, and Carlson (1924)				+		
		Bracewell, Hoyle, and Zilva (1930)				+ to ++		
	Apricots (Fresh)	Morgan and Field (1930) ...	++					
	(Dried)	Morgan and Field (1930) ...	+					
	Avocado (Alligator Pear)	Jansen and Donath (1924)	++					
		Weatherby, Youtz, and Watson (1929)	++					
		Weatherby and Waterman (1928)		++				
		Dickey (1927) ...				— to +		
	Banana ...	Eddy and Kellog (1927) ...	++	+		+++	— to ±	
		Munsell (1924) ...	++					
		Sopp (1924) ...	++					
		Sherman and Munsell (1925)	++					
		Sugiura and Benedict (1919)	+	±		+		
		Acuna (1923) ...		+				
		Eddy (1927) ...		+	++	+++		
		Lewis (1919) ...				++		
		Givens, McClugage, and van Horne (1922)				++		
		Jansen and Donath (1925)				++		
		von Meysenbug (1928) ...				+++		
		Eddy (1929) ...				++		
		Gruninger (1928) ...					±	
		Evans and Burr (1927) ...						+
	Batata (Yellow and Red) (Purple and White)	Jansen and Donath (1924)	++					
		Jansen and Donath (1924)	±					
	Bread Fruit ...	Jansen and Donath (1928)	++					
	Cantaloupe ...	Newton (1928) ...	++	++				
		Sherman (1926) ...		++				

Class of Foodstuffs.	Individual Foodstuff.	References.	VITAMIN					
			A	B ₁	B ₂	C	D	E
FRUITS— continued.	Cantaloupe —continued.	<i>Georgia Agri. Expt. Stat. Report</i> (1926-8)				++		
	Cashew Apple	Jansen and Donath (1928)	+					
	Cherries (Canned)	Bailey and Kramer (1928)	++					
		<i>Kansas Agri. Expt. Stat. Report</i> (1926-8)		++				
	Chicos (Sapo- dillas)	Jansen and Donath (1924)	+					
		Embrey (1923)				++		
	Cloudberries ...	Holst and Frölich (1912)				+		
	Cocum... ..	Chick, Hume, and Skelton (1919)				Low		
	Cranberry ...	Holst and Frölich (1912)				Low		
		McLeod and Booher (1930)				Low		
	Currants (Dried)	Chick and Hume (1917) ...		Very low				
	Custard Apple	Jansen and Donath (1928)	—					
		Schlossberg (1929)... ..				±		
	Dates (Dried)...	Smith (1928)	+	++				
		Chick and Hume (1917) ...		Very low				
		McLeod and Booher (1930)				Low		
	Grape Fruit and Juice (Fresh)	Morgan and Chaney (1924)	+					
		Jansen and Donath (1928)	+					
		Osborne and Mendel (1920)		+				
		Sherman (1926)		+				
		Willimott (1926)		+				
		Jansen and Donath (1924)				+++		
		McLeod and Booher (1930)				++		
	(Dried) ...	Osborne and Mendel (1920)		+				
		Givens and Macy (1921) ...				+		
	(Peel)... ..	Willimott and Wokes (1926)	— to +	++				
	(Canned) ...	McLeod and Booher (1930)				++		
	Guavas ...	Embrey (1923)				++		
	Lemon and Juice (Fresh)	Osborne and Mendel (1920)	almost—	++				
		Morgan (1923)	±					
		Morgan and Chaney (1924)	±					
		Sherman (1926)		++				
		Chamberlain and Vedder (1911)		±				

Class of Foodstuffs.	Individual Foodstuff.	References.	VITAMIN					
			A	B ₁	B ₂	C	D	E
FRUITS —continued.	Lemon and Juice (Fresh)— <i>contd.</i>	Holst and Frölich (1912)				+++		
		Smith (1918, 1919) ...				+++		
		Chick, Hume, Skelton, and Smith (1918)				+++		
		Harden and Zilva (1918) ...				+++		
		Chick, Hume, and Skelton (1919)				+++		
		Davey (1921) ...				+++		
		Delf (1925) ...				+++		
		Jansen and Donath (1925)				+ to +++		
		Zilva (1924-8) ...				+++		
		(Dried) ... Osborne and Mendel (1920)		++				
		Harden and Zilva (1918) ...				+++		
		Bassett-Smith (1920) ...				+++		
		Givens and Macy (1921) ...				+++		
		(Peel)... Willimott and Wokes (1927)	+					
		Willimott (1926) ...		+				
		Willimott and Wokes (1926)				almost—		
	Limes and Juice	Jansen and Donath (1928)	+					
		<i>Med. Res. Council Report</i> (1924-8)	D	+				
		Chick, Hume, and Skelton (1918)				++		
		Smith (1918, 1919) ...				+ to ++		
		Robison (1919) ...				++		
		Davey (1921) ...				++		
	Mango ...	Jansen and Donath (1928)	+					
	(Dried) ...	Chick, Hume, and Skelton (1919)				±		
	Mangosteen ...	Jansen and Donath (1928)	—					
	Mulberry ...	Holst and Frölich (1912)				+		
	Orange and Juice	Morgan (1923) ...	+					
		Byfield, Daniels, and Loughlin (1920)	++	+				
		Osborne and Mendel (1920)	Low	+				
		Morgan and Chaney (1924)	+	+				
		Willimott (1928) ...	++	++				
		Harden and Zilva (1918) ...		+				

Class of Foodstuffs.	Individual Foodstuff.	References	VITAMIN					
			A	B ₁	B ₂	C	D	E
FRUITS— <i>continued.</i>	Orange and Juice — <i>continued.</i>	Sherman (1926)		+				
		Chick and Rhodes (1918)...				+++		
		Chick and Dalyell (1919) ...				+++		
		Harden and Robison (1919, 1920)				+++		
		Delf (1920, 1921, 1925) ...				+++		
		Davey (1921)				+++		
		Wright (1921)				+++		
		Goss (1925)... ..				+++		
		Priston (1926)				+++		
		Eddy (1929)				+++		
		Willimott (1928)					almost—	
		Evans and Burr (1927) ...						± to +
	(Dried) ...	Osborne and Mendel (1922)	+					
		Osborne and Mendel (1920)		+				
		Givens and McClugage (1919)				+++		
		Harden and Robison (1920)				+++		
	(Irradiated)...	Maslow, Shelling, and Kramer (1926)					++	
	Orange Mar-malade	Delf (1921)				Very low		
	Orange Peel ...	Willimott and Wokes (1927)	+	+				
		Osborne and Mendel (1920)		+				
		Hess and Fish (1914) ...				+		
		Hess and Unger (1918) ...				+		
	Papaya ...	Jansen and Donath (1924)	+					
		Miller (1926)	++	+		+++		
		Acuna (1923)		+				
		Delf (1921)				+++		
	Peaches ...	Kohman, Eddy, Carlsson, and Halliday (1926)	+to++			+to++		
		(Canned) <i>Kansas Agri. Expt. Stat. Report (1926-8)</i>		+		+to++		
		(Dried) Eckman (1922)				+		
		Morgan and Field (1929, 1930)	+			+		
	(Raw) ...	Newton (1928)	+	+				
		Morgan and Field (1930)...	++					
		Sherman (1926)		+				

Class of Foodstuffs.	Individual Foodstuff.	References.	VITAMIN					
			A	B ₁	B ₂	C	D	E
FRUITS —continued.	Peaches (Raw) <i>continued.</i>	Delf (1921)				++		
		Kohman, Eddy, Carlsson, and Halliday (1926)				++		
	Pears	Craven and Kramer (1927)				— to +		
	(Canned) ...	<i>Kansas Agri. Expt. Stat. Report</i> (1926–8)				— to +		
	(Raw) ...	Osborne and Mendel (1920)		+ (low)				
		<i>Kansas Agri. Expt. Stat. Report</i> (1926–8)		+ to ++				
		Craven and Kramer (1927)				++		
	Persimmon (Chinese)	Sherman (1929)	+					
		<i>Med. Res. Council Report</i> (1924–8)		—		+		
		Embrey (1923)				+		
	(Japanese) ...	Iwasaki (1927)				+		
		Hsu (1928)				±		
		Tilt, Hubbell, and Inman (1930)		±				
	(Philippine)	Jesus, F. de (1927) ...		— to +				
	Pimento (Can- ned)	McLeod and Booher (1930)				+		
	Pineapples (Canned)	Miller (1924)	++	++				
		Miller (1925)				++		
	(Raw) ...	Miller (1924)	++	++				
		Delf (1921)				++		
	Plantain ...	Cook (1927)	++	++				
		Cook and Quinn (1928) ...	++	++				
	Pomegranate ...	Sherman (1929)				++		
	Prickly Pear (Cactus)	Delf (1921)				+		
	Prunes (Dried)	Morgan and Field (1930) ...	+					
		Sherman (1926)	++	++				
		Hess and Unger (1918) ...		+		—		
		Shimoda, Fujimaki, and Saiki (1927)		+		—		
	(Fresh) ...	Osborne and Mendel (1918)		+				
	Raisins ...	Dutcher and Outhouse (1923)	—			—		
	Raspberries and Juice (Dried) ...	Holst and Fröhlich (1912)				+		
		Givens and Macy (1921) ...				—		

Class of Foodstuffs.	Individual Foodstuff.	References.	VITAMIN					
			A	B ₁	B ₂	C	D	E
FRUITS —continued.	"Red Fruit" (<i>Crataegus pinnatifida</i>)	Hsu (1928)				+		
	Santol	Jesus (1927)		+				
	Strawberries (Raw)	Kohman, Eddy, and Halliday (1928)	±	+		+++		
	(Canned) ...	Kohman, Eddy, and Halliday (1928)				+++		
	Tamarind ...	Chick, Hume, and Skelton (1919)				+(low)		
	Tangerines ...	Delf (1921)				+++		
MISCELLANEOUS.	Beer (Pale) ...	Harden (1918, 1924) ...		—		—		
		<i>Med. Res. Council Report</i> (1924-8)		0 to +		—		
	(Dark) ...	Scheunert and Schieblich (1927)		Low				
	(Kaffir) ...	Delf (1921)				+		
	Chocolate (Irradiated)	Reid (1929)... ..					++	
		Krasso (1929)					++	
	Coffee (Raw) ...	Schwarz and Sieke (1930)					+	
	Diatoms (Marine)	Hjort (1922)	+++					
		Jameson, Drummond, and Coward (1922)	+++					
		Leigh, Clare (1927) ...	—	—		—	—	
	Glucose ...	<i>Med. Res. Council Report</i> (1924-8)	—	—		—		
	Heliocitin ...	Steudel (1929)	+				++	
	Honey ...	Kifer and Munsell (1929)	—	—		—		
		Hawk, Smith, and Bergheim (1921)		—				
		Caillas (1926)		+				
		Hoyle (1929)		—				
		Faber (1920)				—		
	Lactose ...	McCollum and Davis (1915)		—				
		Harden and Zilva (1918) ...				—		
		Cohen and Mendel (1918)				—		
	Molasses ...	Sherman (1926)	—			—		
		Nelson, Heller, and Fulmer (1925)		+				
	Seaweeds (<i>Ulva</i> , <i>Cladophora</i>)	Coward and Drummond (1921)	++					
	(<i>Polysiphonia</i>)	Hjort (1922)	++					

Class of Foodstuffs.	Individual Foodstuff.	References.	VITAMIN					
			A	B ₁	B ₂	C	D	E
MISCELLANEOUS —continued.	Seaweeds (Polysiphonia) —continued.	Coward and Drummond (1921)	+					
	(Chondrus crispus)	Coward and Drummond (1921)	—					
	Spruce Infusion	Appleton (1921, 1922) ...				+		
	Starch ...	Drummond (1919)... ...	—					
		<i>Med. Res. Council Report</i> (1924-8)	—					
	(Corn) ...	Evans and Burr (1928) ...			+			
	(Irradiated) ...	Steenbock, Black, Nelson and Hoppert (1925)					+	
	Sugar ...	<i>Med. Res. Council Report</i> (1924-8)	—	—		—		
	(Cane) ...	Nelson and Jones (1930) ...		Low				
	Tea (Black) ...	<i>Med. Res. Council Report</i> (1924-8)		—				
		Mattill and Pratt (1928) ...				+		
	(Green) ...	Miura and Tsujimura (1928)				+		
		Munsell and Kifer (1929)				—		
		Mitchell (1929) ...				—		
		Munsell and Miller (1930)				—		
	Wine ...	Randoin and Portier (1922)		+				
		Randoin (1928) ...		±	+			
	Yeast (Moist)	Drummond and Coward (1920)	—					
		Nelson, Heller, and Fulmer (1923)	—					
		Luce and Maclean (1925)...	— to +					
		Hume, Smith, Smedley, and Maclean (1928)	— to +					
		Cooper and Funk (1911) ...		+++				
		Cooper (1912, 1914) ...		+++				
		Funk (1913) ...		+++				
		Willcox (1916) ...		+++				
		Seidell (1917) ...		+++				
		Drummond (1917)... ...		+++				
		Simonnet (1921) ...		+++				
		Williams (1921) ...		+++				
		Daniels (1922) ...		+++				

Class of Foodstuffs.	Individual Foodstuff.	References.	VITAMIN					
			A	B ₁	B ₂	C	D	E
MISCELLANEOUS —continued.	Yeast (Moist) —continued.	Kennedy and Palmer (1922)		+++				
		Heller (1923) ...		+++				
		Scheunert and Schieblich (1929)		+++				
		Goldberger, Wheeler, Lillie, and Rogers (1926)			++			
		Chick and Roscoe (1927) ...			++			
		Hassan and Drummond (1927)			++			
		Sherman and Axtmayer (1927)			++			
		Bloxsom (1929) ...			++			
		Munsell (1929) ...			++			
		Hess (1916) ...				—		
		Chick and Hume (1917) ...				—		
		Hume, Smith, and Smedley Maclean (1928)					—	
		Drummond and Coward (1920)	—					
		Nelson, Heller, and Fulmer (1923)	—					
		Pharmaceutical Society of Great Britain (1927)		+ to ++				
		Schittenhelm and co-workers (1927)		++				
		Willimott and Wokes (1928)		+ to ++				
		Chick and Roscoe (1928) ...			+			
		Williams and Waterman (1929)			+			
		Cenley (1930) ...			+			
		Chick and Hume (1927) ...				—		
	Yeast Extract	Drummond and Coward (1920)	—					
		Cooper (1912, 1914) ...		+++				
		Chick and Hume (1917) ...		+++				
		Plimmer (1928) ...		+ to +++				
		Kennedy and Palmer (1928)		+ to +++				
		Willimott and Wokes (1928)		+++				
		Randoin and Lecoq (1926)			+ to +++			

Class of Foodstuffs.	Individual Foodstuff.	References.	VITAMIN					
			A	B ₁	B ₂	C	D	E
MISCELLANEOUS —continued.	Yeast Extract —continued. (Irradiated) ...	Hassan and Drummond (1927)			+ to ++			
		Kennedy and Palmer (1928)			++			
		Chick and Hume (1917) ...				—		
		Hess (1927)... ..					++	
		Hume, Smith, and Smedley Maclean (1928)					++	
		Kirsch (1928)					++	
		Wachtel (1929)					++	
		Kon and Mayzner (1930)...					++	

ADDENDUM

The following information came to the notice of the author too late to be incorporated in the general body of the text. It is therefore added as an addendum.

(1) **SYNTHESIS OF VITAMIN A BY NITZSCHIA CLOSTERIUM** (p. 14).—The results of Ahmad (1930) confirm the view that these diatoms are the ultimate source of vitamin A in fish liver oils, and suggest that a similar change takes place in the cod, giving rise to the stores of colourless vitamin A present. They also explain why the liver oils of fish may be pale in colour and at the same time rich in vitamin A.

(2) **VITAMIN MINERAL RELATIONSHIPS** (p. 23).—It is suggested by Chidester (1931) that iodine is of importance in the treatment of vitamin deficiency. In 1930, Chidester and Speicher stated that “linoleic acid, in combination with ferrous iodide, induced growth, and the ferrous iodide cured the xerophthalmia.” They also suggest that possibly iodine enables intestinal bacteria to make vitamin A.

(3) **SUSCEPTIBILITY TO INFECTION DUE TO MIXED VITAMIN DEFICIENCY** (p. 44).—It is pointed out by Wright (1931) that keratomalacia may not be, as hitherto believed, a pure vitamin A deficiency. Although crude cod-liver oil produces beneficial effects, a preparation of pure vitamin A was not so efficacious. Wright suggests that there is no proof that it is the fat-soluble vitamin A in cod-liver oil alone which has a specific effect on keratomalacia, and that cod-liver oil contains also vitamins B, C, and D. Other food deficiencies, of which the most common, in Madras, is that of vitamin B₁ and vitamin B₂, are necessarily associated with the vitamin A deficiency, and Wright suggests that it requires an initial multiple vitamin deficiency to account for the whole picture of keratomalacia.

(4) **QUANTITATIVE ESTIMATION OF VITAMIN A** (p. 50).—In the factors affecting the accuracy of the biological test for vitamin A, the importance of the amount of yeast in the basal diet is further confirmed by Norris and Church (1930).

(5) **QUANTITATIVE ESTIMATION OF VITAMIN B₁** (p. 53).—A colorimetric method of estimating the amount of antineuritic vitamin in rice is suggested by Spruyt (1930), though its value is uncertain until confirmatory experiments have been undertaken. He extracts the rice with an acid mixture containing norite, the filtrate precipitated and centrifuged with phospho-tungstic acid, and the precipitate from this reduced with zinc until a final brownish-

red colour is obtained which can be matched with a standard glass slide. Spruyt states that the animal test curve and the colorimetric curve follow each other closely.

(6) **VITAMIN A AND CAROTIN**—(a) **Identity of the Two Substances** (p. 67).—Moore (1931) now suggests that the effects following the ingestion of carotin are not due to its identity with vitamin A, but that carotin is a precursor of vitamin A, and leads to its production within the animal body.

This supposition is confirmed by the work of Wolf, Overhoff, and van Eckelen (1930). They were able to separate vitamin A and carotin by shaking a petrol ether solution of the two with 90 per cent. alcohol. Vitamin A goes into the alcohol layer, while carotin remains in the petrol ether.

(b) **Absorption Band of Carotin** (p. 69).—Capper (1930) has also found no absorption band at $328\ \mu\mu$ in carotin. Unless, as suggested by Rosenheim and Webster, the band at $328\ \mu\mu$ is considered to be definitely not characteristic of vitamin A, Capper suggests that vitamin A must exist in at least two forms, or that the vitamin is synthesised *in vivo* from carotin.

(c) **Storage of Carotin** (p. 70).—Moore (1931) believes that carotin is not stored in the liver, but that carotin in the diet leads to a proportionate appearance in the liver fat of vitamin A as such.

(7) **VITAMIN A AND CHOLESTEROL** (pp. 71 and 74).—It is suggested by Seel and Danmeyer (1931) that cholesterol rather than carotin may be the precursor of vitamin A. In contrast to Rosenheim's (1927) results, these workers found the blue colour produced by the interaction of cholesterol and arsenic trichloride permanent if olive oil instead of cod-liver oil were used as a solvent. Seel believes with Takahashi that the vitamin A formed on oxidising cholesterol is of a sterol nature, and that it is a very labile partial oxidation product of cholesterol.

(8) **SOLUBILITY OF VITAMIN A** (p. 73).—Contrary to the statement in the text that vitamin A cannot be extracted from plant tissues by lipoid solvents, recent work by Quinn, Hartley, and Derow (1930) shows that vitamin A from dried tomatoes, carrots, and broccoli is readily soluble in pea-nut oil.

(9) **STABILITY OF VITAMIN A**—(a) **Rancid Fats** (p. 83).—Contrary to the experience of Powick and Fridericia (p. 85) that vitamin A is inactivated by rancid fat, Quinn, Hartley, and Derow (1930) found no rapid or marked destruction of vitamin A in spinach to which rancid butter fat was added.

(b) **Pasteurisation** (p. 84).—According to Pratt (1930), the pasteurisation of milk either in nickel or glass containers has no effect upon its vitamin A content.

(c) **Preservation** (p. 84).—The storage of dried ground spinach in mason jars in diffused light for periods extending up to 15 months is estimated by Quinn, Hartley, and Derow (1930) to produce 70 per cent. of destruction of vitamin A.

(d) **Ultra-violet Rays**.—The above workers state that vitamin A in petroleum extracts of carrots showed destruction when irradiated for 3 to 5 hours by a mercury-quartz lamp.

(10) **KERATOMALACIA AND VITAMIN A** (p. 87).—According to Wright (1931), keratomalacia is not necessarily a specific vitamin A deficiency, though he states that "it seems more than likely that the principal initial factor is an A-deficiency, and that certain of the prominent clinical features are due to the effects of this deficiency on important tissues and organs of the body, such as the epithelial surfaces of the integument and mucous membranes and their glandular diverticulæ. . . . The primary pathological picture in the keratomalacia deficiency-complex is one of degeneration and wasting of the epithelial surfaces. . . . The clinical impression conveyed is that the organismal attack in human keratomalacia is a late secondary association." He suggests that vitamin B deficiency may play a part in its protean manifestations.

The treatment of ophthalmic diseases, with a combination of a vitamin A preparation and ultra-violet light, has, according to Cardell (1930), had good results only in conditions occurring superficially or in the anterior segment of the eye, such as phlyctenular ophthalmia, hordeola, episcleritis, corneal ulcers, and tuberculous iritis. The more deeply seated lesions have not shown very satisfactory improvement.

(11) **SPECIFIC TISSUE CHANGES IN VITAMIN A DEFICIENCY** (p. 90, *seq.*).—According to Henrikson (1929), the cell degeneration in vitamin A deficiency is complete and progressive, a microscopic feature being drying of tissue. For regeneration, the minimum dose is five-thousandths mgrms. of cod-liver oil per 100 grms. body-weight of rat. During regeneration there is replenishing of the tissue with liquid and resorption of injured tissue components.

(12) **INTESTINAL LESIONS IN VITAMIN A DEFICIENCY** (p. 92).—According to Tilden and Miller (1930), vitamin A deficiency in the monkey manifests itself chiefly by gastro-intestinal lesions. Xerophthalmia, sinusitis, mastoiditis, or abscesses of the tongue were not found in these animals, though epithelial metaplasia of the respiratory and genito-urinary tracts was a fairly constant manifestation.

(13) **SUSCEPTIBILITY TO INFECTION IN VITAMIN A DEFICIENCY** (p. 97).—Recent experiments of Topley, Greenwood, and Wilson (1931) seem to throw doubt on the alleged specificity of vitamin A in raising the resistance of bacterial infection. *B. ærtrycke* infection was introduced into populations of mice fed in various ways and the course of the epidemics observed. The control animals, on a stock diet of whole oats, water, and pasteurised milk, showed a significantly lower mortality than any of the other groups. The group which received the addition of a vitamin A concentrate made from liver appeared to be unfavourably affected, as did those which received an excess of fat, butter, or lard. The addition of cabbage, carrots, or mangolds to the normal diet did not lessen the severity of the epidemic which followed exposure to infection. The addition of carrots indeed appeared to act unfavourably.

(14) **VITAMIN A AND RESPIRATORY TRACT INFECTIONS** (p. 98).—The later experience (1931) of Mellanby in the value of vitamin A as a therapeutic agent in acute infective conditions leads him to conclude that its effect is limited. He believes it probable that it will be more effective as a prophylactic against infection. He also believes that clinical trials now proceeding on the prophylactic effect of vitamin A on such infections as the common cold, sore throat, etc., will show that diet will not prevent the common cold completely, but that it will reduce the incidence of the infection and shorten the period of convalescence by limiting the spread of bacterial infection down the bronchi, and so reducing or eliminating the coughing stage.

(15) **VITAMIN A AND PYORRHŒA ALVEOLARIS**.—The second part of Mrs. Mellanby's work (1930) on the connection between diet and dental caries shows that while vitamin D is necessary for the normal development of the hard structures of the teeth, vitamin A is responsible for the normality of the periodontal tissues. Her conclusion is that "a deficiency of vitamin A in the diet leads to hyperplasia of the subgingival epithelium, and to its subsequent invasion by pathogenic organisms."

(16) **NERVE DEGENERATION IN VITAMIN A DEFICIENCY** (p. 102).—A further effect of vitamin A deficiency is reported by Hughes, Lienhardt, and Aubel (1929), in the appearance of marked nervous symptoms in pigs, chickens, and cows—impaired vision, inco-ordination, and spasms. In the advanced stages portions of the spinal cord, optic, sciatic, and femoral nerves showed degeneration of the nerve bundles.

(17) **STORAGE OF VITAMIN A** (p. 103).—It is suggested by Poulsson (1930) that the vitamin reserves of the male differ from those of the female. He has found that the subcutaneous fat of the cow is fifteen times richer in vitamin A than that of the bull, also the subcutaneous fat of a woman aged 49 was twice as potent as that of a man aged 25. On the basis of these facts, and on the known comparative immunity of women from hemeralopia, Poulsson assumes.

that the superiority of the female fat in vitamin A declines as the woman passes the child-bearing age, when the vitamin reserves are required no longer.

A study of the localisation of vitamin A in retinal and brain tissue by Holm (1929) seems to indicate that while brain tissue contains very little vitamin A, the retina is comparatively rich, the vitamin being concentrated in the layer of rods and cones.

(18) **ABSORPTION OF VITAMIN A** (p. 103).—Following the researches of Koehne and Mendel into the utilisation of vitamin A when administered by ways other than the gastrointestinal tract, Rowntree has studied the fate of vitamin A in the body. Apparently vitamin A is not excreted through the urine of young children and infants even when generous amounts are fed. A marked faecal loss of vitamin A occurred, the largest loss with the largest intake, and also the largest retention on the largest intake.

(19) **UNSATURATED FATS IN THE TREATMENT OF VITAMIN DEFICIENCIES** (p. 109).—Following the experiments of Burr and Burr (1929) with "essential fatty acids," Chidester (1931) reports good results in the treatment of xerophthalmia with linoleic acid, in combination with ferrous iodide. He states that in those animals which consume the linoleic acid combination there is apparently an ability to synthesise vitamin A.

(20) **VITAMIN A IN FOODSTUFFS**—(a) **Butter** (p. 112).—Contrary to the statement of Rosenheim and Webster (1927) that butter contains one-tenth to one-twentieth as much vitamin A as cod-liver oil, Morton and Heilbron (1930) have calculated that the unsaponifiable matter of New Zealand butter contains about six times the quantity of vitamin A in good cod-liver oil. They have based their estimation on spectroscopic examination, comparing the intensity of the absorption bands with those given by pure carotin and by vitamin A concentrates. By measuring the intensity of these bands, and those given in the antimony trichloride test, they claim to have made an approximate estimation of the amounts of vitamin A and of carotin in the butter.

New Zealand butter contained about 0.5 per cent. of carotin, and Danish about 0.1 to 0.15 per cent.

(b) **Maize** (p. 116).—Hauge (1930) confirms the fact that vitamin A is lacking in the grains of hybrid red maize with pure white endosperm, even when grown on the same ears as those possessing grains with yellow endosperm. The colour of the pericarp is stated to have no effect on the vitamin A content of maize, the vitamin being associated always with the yellow endosperm.

(21) **SOURCE OF VITAMIN D** (p. 125).—Following the discovery that vitamin D is present in many fungi, members of the genus *Mycobacterium* have been investigated by Prickett and Massengale (1931).

Contrary to expectation, since these species contain relatively large amounts of lipoids, none of the cultures (including *M. tuberculosis*, *M. avium*, *M. lepræ*, *M. smegmatis*, *M. beroliensis*, and *M. phlei*) grown on 5 per cent. glycerol agar contained ergosterol when tested spectroscopically. These same cultures showed some stimulation of growth from the addition of ergosterol (activated and non-activated) in concentrations up to 1.25 mgrm. per slant during the first two weeks of incubation, with more intense pigmentation of the growths. No difference was found, however, on continued incubation.

(22) **CONCENTRATION OF VITAMIN D** (p. 129).—Crystals, which they suggest may be a relatively pure specimen of one of the antirachitic substances formed during the irradiation of ergosterol, have been prepared by Askew, Bourdillon, and co-workers, by the distillation of ergosterol. Fractions of the distillate were condensed at different temperatures, and one of the more volatile fractions was redistilled and crystallised from aqueous alcohol. The crystals were powerfully antirachitic, and gave an absorption spectrum similar to that previously attributed to vitamin D.

These workers point out a second possibility that these crystals, though probably not pure vitamin D, may consist of an inactive substance contaminated with extremely active vitamin, or of crystalline vitamin forming mixed crystals with an inactive substance.

(23) **THE METABOLISM OF ERGOSTEROL** (p. 131).—The distribution of ergosterol in the body has been further studied by Page (1931). He gave large doses of viosterol to rabbits over periods varying from 36 to 164 days. He found that the brain, adrenals, liver, and to a slight extent the kidneys, contained ergosterol, the other organs examined showed none. The bones apparently do not store ergosterol even after prolonged overdosage.

(24) **STABILITY OF VITAMIN D DURING STORAGE** (p. 132).—According to Norris, Heuser, and Wilgus (1929), the vitamin D in cod-liver oil in a feed mixture for chickens showed material destruction when the feed mixture was kept in sacks at room temperature for 12 to 16 weeks. The quantity of vitamin D destroyed varied directly with the length of storage.

(25) **TRANSMISSION OF ULTRA-VIOLET LIGHT THROUGH CELOGGLASS** (p. 139).—The value of celoglass, which consists of cellulose acetate on a wire mesh, is emphasised by Wyman and co-workers (1930), and Wilder and Vack (1930). Experiments carried out by the former workers in solaria equipped with such glass showed that even winter sunshine transmitted through it had an antirachitic effect upon children with rickets.

(26) **RACHITIC BONE IN RATS** (p. 142).—Park (1930) states that one of the factors in the production of "cupping" of the diaphysis is a delayed assimilation of the cartilage cells, more marked in the central portion of the bone. Simultaneous with the proliferative change in the cartilage there is a very gradual and even failure in the lime salt deposit, and histological changes can be detected in the bones at the end of the first week when the serum phosphorus is still within normal limits.

(27) **ERROR OF PHOSPHORUS METABOLISM IN RICKETS** (p. 145).—The view that a disturbance of phosphorus metabolism is more important than that of calcium in rickets is supported by Rominger and co-workers (1930), Compère (1930), and Macciotta (1930). The former observers state that at the commencement of florid rickets and of the healing state, the alteration in P metabolism always occurs and proceeds to a definite degree, previous to the alteration in Ca metabolism, and they therefore consider the derangement of P metabolism the primary factor.

Compère finds that though phosphorus alone is not sufficient treatment to produce healing in severe cases of rickets, the combination of cod-liver oil and phosphorus produces more prompt and more certain healing than does cod-liver oil alone.

Macciotta bases his view on the fact that when pregnant animals are deprived of phosphorus, the offspring show histological, radiographic, and morphological manifestations of rickets.

Rominger and co-workers also state that the administration of irradiated ergosterol primarily affects the P and not the Ca metabolism, and this statement is confirmed by Warkany (1930), who finds that the blood phosphate in rabbits fed 10 c.c. vigantol increases immediately after feeding.

(28) **VITAMIN D AND THE PARATHYROIDS** (p. 152).—Although Greenwald has reported that cod-liver oil is ineffective in preventing tetany in thyro-parathyroidectomised dogs, it is antirachitic, according to Pappenheimer (1930), in the absence of either or both parathyroids and the thymus. Activated ergosterol had, in one of his experiments, the same effect, one drop daily preventing rickets in rats after removal of both thymus and parathyroids, while the inoperated controls developed rickets.

(29) **ANTIRACHITIC EFFECT OF WINTER SUNSHINE** (p. 155).—The seasonal variation of rickets pointed out by Tisdall and Brown as due to the diminished potency of winter sunshine in Toronto is not confirmed, so far as Colorado is concerned. Lewis and co-workers (1931)

have found no marked difference in the antirachitic effect of winter and summer sunshine in Colorado. They explain the difference between their results and those of Tisdall and Brown on the ground that, owing to the high percentage of winter sunshine, relatively short-wave radiations reach the earth all the year round, also that the atmosphere is relatively free from moisture and smoke.

(30) **TREATMENT OF RICKETS WITH ULTRA-VIOLET LIGHT** (p. 155).—Small doses of quartz-lamp irradiation are stated by Vollmer (1930) to produce results quite as good as those obtained with larger doses. He considers the minimal effective dose a weekly irradiation of from 4 to 5 minutes on the chest and back at a distance of 80 cms. He suggests that in many cases too large quantities are applied which not only overburden the child, but are also a waste of time and electric power.

(31) **TREATMENT OF RICKETS WITH COD-LIVER OIL** (p. 158).—In comparing the value of cod-liver oil with other methods of rickets therapy (irradiation of the skin and administration of irradiated ergosterol therapy), Macrae (1930) considers cod-liver oil the method of selection in hospitals and welfare centres, both on economic grounds, and because the results obtained are equally satisfactory.

(32) **PRENATAL PROPHYLAXIS IN RICKETS** (p. 158).—It is stated by Macciotta (1930) that deficiency of the antirachitic factor in the maternal diet, either during pregnancy or during nursing, causes the development of rickets in the offspring. He concludes that the prophylaxis of rickets in infants should begin during the foetal period, and be combined through the nursing period, by adjustment of the maternal diet.

(33) **TOXIC EFFECTS OF COD-LIVER OIL** (p. 160).—It is suggested by Norris and Church (1930) that the toxic effects of cod-liver oil may be due to the presence of iso-amylamine, which constitutes one-third of the bases present. They found that small doses of iso-amylamine, in amounts found in cod-liver oil, result in paralysis, convulsions, and lack of growth, and that this toxicity may be neutralised or prevented by adding yeast.

(34) **TREATMENT OF RICKETS WITH IRRADIATED MILK** (p. 161).—Milk irradiated by the Jesoniek-Brenner-Milch method is stated by Hentschel and Fischer to be a more potent antirachitic agent than milk irradiated by other methods. Healing in florid rickets was evident in 3 weeks and complete after 6, while 2 cases of spasmophilia were rapidly cured.

(35) **IRRADIATED FOODSTUFFS** (p. 161).—(a) **Butter**.—The conditions necessary to secure the maximum activation of butter-fat have been investigated by Steenbock and Wirick (1930). The distance of exposure from the lamps, two types of which were used (Alpine Sun and Cooper-Hewitt mercury vapour), was kept at 18 inches, and the temperature was not allowed to exceed 50° F. Using a film of fat 160 mm. thick, most of the activation occurred during the first 10 minutes, or less. The second 10 minutes caused some further activation, but the effect of the third was questionable.

A further 2 hours' activation caused no increase, and 16 hours caused destruction. The vitamin D content of the butter-fat used was low—5 per cent. of the rachitogenic ration (or 400 mgrms. per rat daily) did not allow normal bone production. Irradiation produced a potency such that 20 mgrms. of irradiated butter-fat was equal to 400 mgrms. of non-irradiated. Cod-liver oil was, however, forty times as potent.

Storage did not destroy the potency of the irradiated butter-fat, and injudicious irradiation produced no deleterious effects.

(b) **Yeast**.—The effect of feeding irradiated yeast to milking cows has been investigated by Hart, Steenbock, Kline, and Humphrey (1930). They found that the milk increased in antirachitic potency, showing that vitamin D was absorbed into the blood, but that there was no positive influence in improving the calcium assimilation. The Ca and P content of the blood and the milk remained unchanged during the period of feeding of the yeast.

(36) **IRRADIATED ERGOSTEROL (IN MILK) IN TREATMENT OF RICKETS** (p. 168).—Hentschel and Fischer (1930) describe an emulsion of irradiated ergosterol ("Vitana-Emulsion") which, when added to milk in the proportion of 5 c.c. to 1 litre, produced a therapeutic effect equal to that obtained by the present reduced dose of standardised vigantol.

(37) **OTHER EFFECTS OF VIGANTOL ADMINISTRATION** (p. 171)—(iii) **Fatty Infiltration of Organs.**—According to Haendel and Malet (1930) guinea-pigs which had received two doses of 10 c.c. vigantol per kgm. body-weight showed marked fatty infiltration of the liver, heart, and spleen, while the epithelial cells of Henle's loop in the kidney showed many cells containing fat and lipoid droplets.

(iv) **Lesions of the Muscles.**—Loss of weight and muscular atrophy, with crippling of the hind-legs, then the fore-legs, and finally of the neck muscles, is stated by Slauek (1929) to occur after administration of vigantol.

(v) **Antagonisation of Growth of Transplanted Tumours.**—A rather uncertain conclusion is reached by Caspari and Ottenssooser (1930), that vigantol antagonises the stimulating effect of a rachitogenic diet on the growth of a transplanted tumour in mice (Ehrlich, No. 5, mouse carcinoma). They state that it is not certain whether its vitamin D or its olive oil content was responsible for this antagonism.

(38) **TOXIC EFFECTS OF LARGE DOSES OF IRRADIATED ERGOSTEROL** (p. 173)—(a) **Kidney Lesions.**—The calcification of the tubules and glomeruli signalled by other workers after the administration of ergosterol is confirmed by Spies and Glover (1930), who observed also sclerosis and hyalinisation of the vessel walls and thickening of the basement membranes of the tubules and glomerular capsules, with extensive subepithelial deposits of hyalin in both. There was pronounced atrophy of the tubular epithelium. Fat deposition was irregular, but was chiefly found in arteries showing marked calcification, and there were large amounts filling the sites of obliterated lumina and the areas of necrosis in some of the vessel walls. The kidney lesions were accompanied by the presence of large amounts of albumen in the urine. Pathological changes in the urine of tuberculous children after the administration of vigantol have also been observed by Klausner-Cronheim (1930). She reports the occurrence of albuminuria, red cells, and hyaline casts, which disappeared on cessation of treatment. She states that the uncontrolled use of irradiated fresh milk may produce similar lesions.

(b) **Changes in the Bones.**—Hyperossification after administration of 5 mgrms. of irradiated ergosterol (vigantol) is described in detail by Collazo and Morelli (1929). X-rays showed a form of calcification quite different from that obtained in normal and rachitic rats—a sharp, clear horizontal line of ossification at the junction of epiphysis and diaphysis, indicating uniform and complete calcification. The bony trabeculae of the osteoid zone were large and strong, and extended towards the diaphysis with inhibition of growth. The periosteum was made visible by a similar line of ossification.

Histological examination of the bones showed irregularly disposed cells impregnated with Ca in the zone of cartilage proliferation, with the line of ossification narrowed, indicating a premature ossification accompanying arrest of growth. In the zone of ossification were numerous large trabeculae, arranged in disorderly fashion, and alternating with columns of cartilage.

(c) **Changes in the Blood.**—In the experiments of Spies and Glover (1930) the blood of rabbits receiving fatal doses of irradiated ergosterol showed a rise in the nitrogenous constituents during the last day or two of life, the terminal values for non-protein nitrogen ranging from 67 mgrms. to 222 mgrms. per 100 c.c. as compared with the control figure of 39 mgrms. The urea nitrogen formed at death 65 per cent. of the non-protein nitrogen, whilst in the normal animals it was only 35 per cent. The creatinine increased to a less degree than the non-protein nitrogen, whilst the uric acid never rose to more than twice its original level, and in

some instances showed hardly any rise. The degree of nitrogen retention was in general proportional to the amount of kidney damage seen on histological examination.

(39) **OTHER THERAPEUTIC EFFECTS OF VITAMIN D.** (p. 183)—(a) **Calcification of Teeth.**—The second part of Mrs. Mellanby's work (1930) on the connection between vitamin D and the formation of teeth strengthens her earlier supposition that dental caries is due to lack of vitamin D in the diet rather than, as Sim Wallace has suggested, to the presence of fermentable carbohydrate. She found that even large quantities of glucose do not produce dental decay in dogs, nor does the presence of *Streptococcus mutans* and *Bacillus acidophilus*, though in the case of rabbits whose exposed dentinal tubules are often invaded by bacteria some results were obtained. There appeared, however, to be less tendency to invasion when fat-soluble vitamins were abundant in the diet.

The exposure of the teeth of animals to attrition was also investigated. It was found that the production of secondary dentine in the teeth of puppies in response to attrition was influenced by the vitamin D in the diet. When the diet is rich in vitamin D much well-calcified secondary dentine is formed. A cereal diet deficient in vitamin D is associated with either defective formation of secondary dentine or none at all. "The amount and character of the secondary dentine formed is a measure of the resistance of the living tooth; none is formed in a dead tooth." Investigations on the teeth of rats and rabbits confirmed her conclusions in the case of dogs, and she suggests the probability that "the tooth structure of all mammalia is controlled, as regards calcification, by the same dietetic and environmental factors as are described in Part I of this report."

(b) **Antibacterial Resistance.**—Later experiments by Robertson and Ross (1930) tend to show an increased resistance to infection by *Salmonella muritidis* (which can only be distinguished serologically from *B. enteritidis*). Of 89 rats fed on a ration of non-irradiated "muffets" (whole wheat), only 5 per cent. survived, as compared with 30 per cent. of rats fed on irradiated "muffets."

(c) **Resistance to Metallic Salt Poisoning.**—According to Hoff (1930) the resistance of guinea-pigs to the toxic action of salts of lead or mercury was increased by maintaining the animals on a diet rich in vitamin D, and reduced by feeding on a diet deficient in vitamin D. He suggests that the increased resistance is due to a decrease in the permeability of the cell membranes, since the resistance was found to be counteracted by theophyllin and alcohol, which are known to increase the permeability of the cell membrane. On the basis of these results, Hoff suggests the following points (assuming that the results in animals can be applied to man):

- (1) A vitamin D rich diet may be of value to workers exposed to the risks of poisoning by metallic salts.
- (2) The consumption of alcohol by such workers might be expected to increase the risks of poisoning.
- (3) The toleration to injections of lead in the treatment of malignant growths might be increased by maintaining the patients on a vitamin D rich diet.

(d) **Paget's Disease.**—One case of Paget's disease, with pronounced bony changes, is reported by Delmas-Marsalet (1930), as being favourably influenced by irradiated ergosterol, combined with calcium gluconate and calcium chloride. Recalcification of the inner table of the skull took place, with some reconstitution of the outer table, and calcification of the falx cerebri was taken as an indication for cessation of treatment.

(e) **Adenoids.**—The Report of the Board of Education Committee, on "Adenoids and Enlarged Tonsils" (1931), suggests some connection, though a far from securely established one,

between vitamin D and the formation of adenoids. The conclusions of the investigators were that adenoids were as common among children free from rickets as among those showing some bony signs, but that severe adenoids occurred more frequently among children with two, three, or more signs of rickets than among those showing one sign or no signs.

The figures show also some association between adenoids and widespread dental caries, which is again known to be associated with lack of vitamin D, but the association of the three factors is not clear. The Committee conclude that a deficiency of fat-soluble vitamins, though not predominant, is one of the factors tending to produce an overgrowth of adenoid tissue, but think that there are other undiscovered factors affecting all classes alike which counter-balance or mask the influence of vitamins.

(40) **PALATABILITY OF IRRADIATED FOODSTUFFS.**—According to Reiter (1929), foodstuffs can be activated without changes in odour and taste if the wave-lengths below $280\text{ }\mu\mu$ are completely filtered out.

(41) **VITAMIN D CONTENT OF FISH OILS** (p. 195).—According to the latest researches of Poulsson (1931) many other fish livers, while richer than that of the cod in vitamin A, are poorer in vitamin D. The shark's liver, for instance, contains only about a tenth of the vitamin D found in the liver of the cod. Poulsson suggests that the deficiency of vitamin D in the shark is connected with the cartilaginous nature of its skeleton. In other fish whose skeletons are composed of cartilage and connective tissue rather than bone the content of vitamin D in the liver oil is remarkably low.

(42) **VITAMIN B COMPLEX** (p. 213).—In connection with the third factor of vitamin B complex, described by Williams and Waterman as thermolabile, and necessary for the growth of pigeons but not of rats, Williams (G. Z.) and Lewis (1930) describe a factor present in the residue from whole yeast after vitamins B_1 and B_2 have been removed. This factor (Fraction R), they state, is thermostable, necessary for the growth of rats (like vitamin B_2), but differs from all other similar factors described. They suggest that it may be water-insoluble, since extraction of yeast fails to produce as good growth as whole yeast.

(43) **CONCENTRATION OF VITAMIN B_1 (SEIDELL'S METHOD)** (p. 222).—A method of purification is described by Seidell and Smith (1930) which results in a product curative in a daily dose of 0.05 mgrm. daily, containing 0.0062 mgrm. nitrogen. The "activated solid," prepared from brewers' yeast by adsorption on fuller's earth, was treated in alkali and acid solutions extracting with chloroform and precipitating the filtered acid aqueous solution in acetone. Further treatment consists in separating the sodium chloride, and repeated precipitation, when a powder is obtained containing 7 to 11 per cent. of nitrogen. Seidell and Smith conclude that this highly potent antineuritic substance is probably free from the thermostable factor.

(44) **EFFECT OF PASTEURISATION ON VITAMIN B_1 IN MILK** (p. 228).—According to Pratt (1930) the antineuritic factor in milk is partially destroyed by pasteurisation in either a glass or nickel container. There was no evidence that the nickel was the catalysing agent in the destruction.

(45) **VITAMIN B_1 DEFICIENCY AS A TOXÆMIA** (p. 234).—With regard to the rapid curve of nervous symptoms in polyneuritis, following the administration of antineuritic concentrates, Cowgill and co-workers (1930) point out that the symptoms are made worse by the ingestion of large amounts of water, negating the idea that the symptoms are those of a toxæmia (see Walsh, 1918). Cowgill suggests that such treatment may actually be detrimental in that it washes vitamin B_1 from an already depleted body.

(46) **ABSORPTION OF GLUCOSE AND PROTEIN IN VITAMIN B_1 DEFICIENCY** (p. 236).—Gal (1930) confirms the view of Pierce and co-workers (1929) that the absorption of glucose is decreased in vitamin B_1 deficiency, being only one-third of the normal. Normal absorption

was induced by supplementing the diet with yeast. The absorption of nitrogenous substances was also decreased, being only one-half the normal.

(47) **VITAMIN B₁ AND PROTEIN METABOLISM** (p. 237).—It is stated by Daniels and co-workers (1930) that a high protein diet, combined with vitamin B₁ deficiency, produces over-development of the thyroid and under-development of the thymus in rats. Either addition of the antineuritic vitamin or a change to a low protein diet brought about a return to normality. The administration of amino-acids (glycerine and alanine) known to stimulate metabolism, produced symptoms similar to those of high protein diets. These workers suggest that the increased requirement of vitamin B₁ when high protein diets are given is due to the increased metabolism resulting from the action of certain of the amino-acids contained in the proteins.

(48) **VITAMIN B₁ REQUIREMENTS OF ANIMALS** (p. 238).—According to Bing and Mendel (1929) the albino mouse requires about half as much vitamin B₁ as the rat.

(49) **CHANGES IN THE HEART IN BERI-BERI** (p. 251).—In confirmation of Aalsmeer and Wenkebach's theory that oedema of the heart muscle is the cause of the cardiac failure in beri-beri, Keefer (1930) records a study of the beri-beri heart, in which the changes formed were compatible with this theory. He points out that the patients who develop cardiac insufficiency are those who have least involvement of the nervous system; this he explains on the ground that nerve paralysis prevents the muscular exercise predisposing to cardiac dilatation.

On the other hand, Newcomb (1930) concludes, from the results of chemical analyses of the heart muscle of pigeons on a vitamin B-deficient diet, that the large heart of beri-beri columbarum is not due to water retention.

(50) **VITAMIN B REQUIREMENTS OF THE NURSING YOUNG** (p. 239).—Recent investigations of Sure and Kik (1930), Sure, Kik, and Smith (1930), and Sure, Kik, and Walker (1930) indicate a disturbance of the liver in the nursing young of albino rats suffering from vitamin B deficiency. A marked alteration was found in the glycogen content of the liver. Expressed in the terms of glucose (total reducing sugars), the glycogen content of the controls showed a range of 45 to 56 mgrms. of glucose, while the polyneuritic young showed a range of 0.8 to 6.2 mgrm. of glucose.

A fatty change in the liver was also found in the majority of the nursling rats whose mothers were on a vitamin B-deficient diet. There was an increase in the actual weight of the liver as well as an increase in proportion to the body-weight, suggesting that the condition was a fatty infiltration rather than degeneration. On the other hand, the nurslings suffering from a specific deficiency of vitamin B₁ showed no fatty changes in the liver.

(51) **EXPERIMENTAL POLYNEURITIS—MUSCULAR SYSTEM** (p. 259).—According to Palladin and Epelbaum (1929) the condition of the muscles in polyneuritis in doves differs according to the chronicity of the disease. In chronic cases there is general muscular weakness, no change in the creatine content, an increase in the lactacidogen, and a decrease in the creatine phosphoric acid. In the acute form there is no muscular weakness, an increase in the creatine content, a decrease in the lactacidogen, and a considerable increase in the creatine phosphoric acid.

(52) **CHANGES IN THE PITUITARY IN VITAMIN B DEFICIENCY** (p. 272).—According to Satwornitskaja and Simnitzky (1928) the pituitary in doves in avitaminosis B shows vacuolisation of the cells, with a decrease in intralobular colloid. Droplets of protein appear in the basophil cells, and similar changes, though less marked, appear in the eosinophil cells. In the rat there is an increase in basophils and an apparent increase in eosinophils.

(53) **VITAMIN B₁ DEFICIENCY AND INFANT MORTALITY** (p. 277).—Following Vogt's (1929) suggestion that deficiency of vitamin B₁ in the maternal diet may lead to abortion or premature

death of the infant, Dr. Margaret Balfour (1931) states that the diet of women who have given birth to premature children is deficient in vitamins A, B, and C, with a specially marked deficiency in B. She suggests that vitamin B deficiency may lead either to weakness of the germ cells, a failure of the growth impulse of the foetus, or a premature thinning and separation of the decidual membrane.

(54) **THE VITAMIN B CONTENT OF MALIGNANT GROWTHS** (p. 281).—According to Jackson and Krautz (1929) there is no evidence that malignant tissue contains unusually large amounts of vitamin B. The more recent experiments of Nakahara and Somekawa (1930) indicate that sarcoma and Flexner Jobling carcinoma contain about one-tenth the amount of vitamin B present in normal rat liver.

(55) **VITAMIN B₁ IN EVAPORATED MILK** (p. 289).—According to Daniels and co-workers (1930), lactating rats on a diet of evaporated milk fail to show normal growth. Since the addition of vitamin B₁ to the diet failed to effect an improvement, these workers conclude that the deficiency in evaporated milk is not that of vitamin B₁.

(56) **VITAMIN B IN FOODSTUFFS** (pp. 292 and 293)—**Persimmon**.—Experiments by Tilt, Hubbell, and Inman (1930) indicate that the Japanese persimmon grown in Florida is low in its content of the vitamin B complex.

Sugar Cane.—According to Nelson and Breese Jones (1930) sugar-cane juice is a poor source of vitamin B₁. The juice from the upper portions of cane stalks is richer than juice from the lower portions. Several sugar-cane products, including cane syrup, blackstrap molasses, and cane cream were found to contain negligible amounts of vitamin B.

(57) **ETIOLOGY OF PELLAGRA** (p. 296)—(1) **An Allergic Phenomenon**.—A theory of the etiology of pellagra advanced by Tarantelli (1930) is somewhat reminiscent of that of Raubitschek (see p. 297), but in Tarantelli's opinion the sensitisation of the tissues is due to pigments and toxins produced by insufficiency of the liver, not by the colouring matters of maize.

(2) **An Iron Deficiency**.—The suggestion is made by Bliss (1930) that pellagra is an iron-deficiency disease. He points out that anæmia is a frequent concomitant of the disease, and that foods which are supposed to contain liberal quantities of vitamin B₂ (beef, liver, egg-yolk, yeast) are all iron-containing foods. He also reports encouraging results from the administration of iron intravenously in severe cases, orally in mild cases of pellagra.

(58) **DESTRUCTION OF VITAMIN C IN MILK BY NICKEL** (p. 318).—Following the suggestion of Hess and Unger (1921) that catalytic destruction of vitamin C in milk might be produced by minute quantities of copper in the vessels in which it is heated, Pratt (1930) has undertaken experiments to determine if nickel has a similar destructive effect. He found that though vitamin C was partially destroyed by pasteurisation in either glass or nickel containers there was no evidence that the catalytic action of nickel increased the destruction.

(59) **VITAMIN C AND METABOLISM** (p. 323)—(a) **Nitrogen**.—According to Palladin and Zuwerkalow (1928) the amount of hippuric acid excreted in the urine after the injection of benzoic acid and glycocoll is decreased during scurvy, the diminution being more pronounced as the disease is prolonged.

(b) **Cholesterol**.—The cholesterol content of the blood and adrenals in scorbutic animals is, according to Morelli (1929), finally decreased after an initial increase. The spleen and liver cholesterol in their experiments remained constant.

(60) **THE BLOOD VESSELS IN EXPERIMENTAL SCURVY** (p. 336).—On the basis of the lesions of the blood vessels described by various observers, Dalldorf (1931) has suggested a test which may have value in the diagnosis of scurvy. He measures the degree of scorbutic change in the vessels of animals with experimental scurvy by establishing the amount of pressure required to produce petechial hæmorrhages in the skin. The test shows that the hæmorrhagic diathesis in experimental scurvy develops earlier than any other known sign of the disease, and that it

persists in some degree throughout. The change in resistance of the vessels follows a curve which rises towards recovery during the end of the first week, reaches a peak in the second week, and then falls steadily during the remainder of the course of the disease.

AUTHOR'S SUMMARY AND CONCLUSIONS

THE ORIGIN OF VITAMINS.—Together with the increasing tendency to regard vitamins as inorganic chemical bodies goes the prevalent opinion that their ultimate synthesis from the primary inorganic elements takes place in plant tissue. In the case of the water-soluble vitamins B and C, their synthesis during the growth of plants has been demonstrated with some clearness, chiefly by the work of Heller.

The origin of the fat-soluble vitamins, A and D, is not so universally accepted. The close association of vitamin A with carotin, together with the conclusions of Drummond (1931) that the primary source of the vitamin A in cod-liver oil is the minute green plant life of the sea, would seem to place it with the water-soluble vitamins as an ultimate product of the vegetable world, synthesised before it reaches the animal body. Moore's latest conclusions are, however, that vitamin A is not identical with carotin, nor is carotin stored in the body, but that its ingestion can lead to the production of vitamin A in the animal tissues. A somewhat similar explanation of the occurrence of vitamin D in the body has been given by Bills and others, but Drummond and Hilditch (1931) still consider it unlikely that vitamin D is synthesised by fish, and incline to believe that both vitamins A and D are concentrated from the large bulk of food, inappreciable though its vitamin content may be, which they consume.

MODE OF ACTION OF VITAMINS.—In spite of the numerous observations which prove beyond doubt that vitamin deficiency results in disturbed body function, and that each vitamin produces its own more or less specific disturbance, the precise manner in which the vitamins are related to tissue function is as yet unknown. The only hypothesis which can be held with certainty is that their primary action is upon those cells which are responsible for the metabolism of the body. All secondary disturbances must be held to derive ultimately from the primary disturbance of metabolism due to the avitaminosis. McCarrison's opinion (1931) probably represents all that is at present known on the subject of vitamin action.

"Whatever the specific function of any vitamin may be, vitamins are but links in the chain of essential substances requisite for the maintenance and harmonious regulation of the chemical processes in the tissues, and their action must be considered in connection with metabolism as a whole, *i.e.* in relation to balance of food ingredients in general to organs of digestion, and assimilation, and to endocrine regulation of metabolism. Their deficiency, like that of the other elements and complexes necessary for normal nutrition, leads to depreciation of cellular function; depreciation of cellular function is the foundation upon which disease is built."

VITAMIN BALANCE.—The chief conclusion which emerges from the rather inconclusive evidence on the vitamin-ratio of the diet is that imbalance of vitamins, in common with any other imbalance of diet, decreases resistance to infection. The fact that this decrease of resistance is apparently correlated to a breakdown of the defensive mechanism of the body, as exemplified in the epithelial living membranes and lymphoid tissues, is interesting not only from the point of view of infection, but also from the suggestion, referred to in the text, of Burrows, Jorstad, and Ernst with regard to cancer. If the epithelial systems of the body are in a constant state of ill-health it is not a far cry to that imbalance of growth which exists in cancerous tissues.

NATURE OF VITAMINS.—The numerous attempts to isolate vitamins in a pure form have so far been unsuccessful. Even though their concentration has been carried so far that the

amount of the vitamin-carrying end-product is almost incredibly small, it cannot yet be said that these "essences" of foodstuffs are the actual vitamins themselves. The standard solution of irradiated ergosterol, which is used therapeutically as though it were vitamin D itself, gives a unit of vitamin D corresponding to 0.0001 mgrm. of the ergosterol used in its production, while the vitamin B₁ concentrated from wheat germ by Guha and Drummond is curative in a dosage of 0.005 mgrm.—quantities small enough to give justification to Drummond's opinion (1930) that "it should be only a matter of carrying out the fractionation on a sufficiently large scale to obtain enough of the active substance or substances to establish their nature and constitution." Professor Baly's conception of vitamins, however, if correct, would explain the failure to obtain them as specific chemical substances, since he maintains that they are not compounds of this nature, but merely "high energy compounds" changing their properties with their energy content.

VITAMIN MINERAL RELATIONSHIPS.—Except in the case of calcium and phosphorus with regard to rickets, the investigation of the relation between mineral salts and vitamins has been incompletely investigated. It is possible, indeed, in the author's opinion, highly probable, that a very important aspect of vitamin research will be developed on these lines. A correlation between the mineral level of the blood and the vitamin and mineral intake has not yet been fully established. Such observations as those of Haage and Palmer (1928) showing that the symptoms of vitamin B deficiency can be closely simulated by those of calcium and phosphorus deficiency; of Delbet and Palios (1929) that magnesium assists the animal to synthesise its own vitamins; of McCarrison (1927) that manganese occurs most abundantly in those plants where vitamins are also abundant; and of McCarrison (1930) that the administration of iodine to rats fed on diets deficient in fat-soluble vitamins may actually cause goitre, are sufficient indications of the complexity of vitamin-mineral action.

VITAMINS AND NUTRITION.—Perhaps no result of vitamin research during the last twenty years has been of greater importance than the establishment of the fact that the animal organism cannot remain in a state of optimum nutrition if the food intake is constantly, even though slightly, deficient in accessory food factors. Minor degrees of malnutrition, while less liable to come under the notice of the experimentalists, are of vital interest to the community. During the period of growth especially, it is essential that tissue equilibrium should be preserved. According to Funk, the damage done during early life by deficient nutrition cannot, in the human being, be remedied by the administration of the lacking factors in later life—a statement whose importance to the economists and guardians of public health needs no emphasis. Cramer's observations (1922) on the results of long-continued "vitamin under-feeding" on the health of the stock are confirmed by those of McCarrison (1931). The contrast drawn by the latter between the two communities of rats—the stock animals, healthy, reproducing normally, with no infantile mortality, and the experimental animals, unhealthy, "with crooked spines, distorted vertebræ, and imperfect teeth, and liable to all forms of incident disease," is too remarkable to be disregarded. Indications of lowered vitality, slow growth, defective reproductive powers, poor condition of the skin and fur, loss of appetite and lethargy, rapidly supervene upon even a mild vitamin deficiency.

It must be remembered that while vitamin deficiency alone will produce these conditions of subnormal health, the basal diet which provides the vitamin deficiency is usually also deficient in other respects. Not only the quantity but the quality of the protein is often defective, and it is evident from the work of Reader and Drummond (1926), Hartwell (1928), Jones (1925), Heller (1927), Mottram and Cramer (1927), McCarrison (1927), and others, that the bad results of vitamin deficiency are made much worse if the protein of the diet is unsatisfactory. McCarrison clearly indicates also that a high proportion of cereals and a low proportion of fat increases the injury done by a vitamin poor diet.

"Of all the faulty diets I have used, that composed of white bread, margarine, tea, sugar, jam, preserved meat, and scanty overcooked vegetables—a diet in common used by many people in this country—proved to be one of the worst."

VITAMINS AND BACTERIAL GROWTH.—The hypothesis that vitamins are, if not essential, at least stimulant to the growth of bacteria, at one time received a certain amount of support. Most observers, however, limited their description of the stimulant substances to that of "vitamin-like substances" or "unknown water-soluble substance." The substances used in the culture media were generally extracts of plant or animal tissues known to be rich in vitamins, but not highly concentrated extracts such as "torulin." Even when activated substances, however, prepared after the recognised method of Funk and Dubin, were used, the results were not sufficiently definite to justify a statement that vitamin B itself was the agent which stimulated the growth of bacteria. Two observations, indeed, would seem definitely to negative the theory: that of McLeod and Wyon (1921), that marmite, known to be rich in vitamins, had little or no effect in promoting the growth of pneumococcus and streptococcus, while charcoal, devoid of vitamins, had quite an appreciable effect, is distinctly opposed to the theory that vitamin B at any rate is an essential factor in bacterial culture, while that of Peters, Kinnersley, Orr-Ewing, and Reader (1928) on the growth-promoting factor for *Streptothrix corallinus* finally disposes of the idea that the stimulating substance is identical with either vitamins B₁ or B₂. It cannot therefore be said that the vitamins themselves are necessary for the growth of bacteria. The most definite conclusions can only go so far as to state that substances analogous in action to vitamins can, when present in the culture medium, stimulate the growth of certain bacteria. The same lack of definiteness is apparent in the question of the production of vitamins by bacteria, except in the case of the synthesis of vitamin B in the intestinal canal of the cow. The results of the experiments of Bechdel and co-workers on the occurrence of vitamin B in the milk of cows fed on a vitamin B-deficient diet are considered to show that the peculiar digestive conditions in the rumen of the cow are specially favourable for the synthesis of vitamin B, while those of Fridericia on the phenomenon of refraction point to a similar synthesis of vitamins B₁ and B₂ when the digestion of starch is defective.

DISTURBANCES OF HEALTH DUE TO NON-SPECIFIC VITAMIN DEFICIENCY.—The almost insuperable difficulty with which the worker on general avitaminosis is faced is, of course, that of apportioning the ill-effects produced to the specific vitamin deficiency. There are, nevertheless, besides the general disturbances of health exemplified by McCarrison's observations on "a good diet and a bad one," several very definite consequences of dietaries lacking in more than one vitamin. Perhaps the most important of these are gastro-intestinal disturbances, endocrine disturbances, and lowered resistance to infection. These ill-effects act together, as it were, in a vicious circle, to produce morbid states leading to the incidence of more definite symptoms of disease. The impairment of the digestive processes, brought about by the degenerative changes in the mucosa, neuromuscular mechanism, and secretory glands of the alimentary tract, result in not only failure of absorption of nutritive elements, but also in absorption of toxic products, and loss of the protective mechanism of the bowel wall against bacterial infection.

The disintegration of the endocrine glands, especially the adrenals, is another link in the chain of morbidity, and though the most marked disturbances take place in the absence of vitamin B, lack of any or all of the vitamins leads to depreciation of the endocrine function in governing metabolism.

With regard to the increased susceptibility to infection, it is probable that the fat-soluble vitamins are more nearly concerned than the water-soluble. The incidence of respiratory and alimentary infections in animals fed on diets deficient in these vitamins is so high that

vitamin A especially has been called the "anti-infective vitamin." It might be expected that the immunological reactions of the blood would show some definite changes due to vitamin-deficient diets, but so far the results of observations on this subject have not been indisputable. Werkman (1923) and Zilva (1919) found the immunity mechanisms of deficient animals practically unchanged, though Orr (*Brit. Med. Journ.*, 23rd May 1931) states that "agglutinins and bacteriolysins against *Bacillus typhosus* in rats are reduced on diets deficient in vitamin A and D, or B and E."

QUANTITATIVE ESTIMATION OF VITAMINS.—The estimation of the amount of vitamins in foodstuffs by the biological method has been brought to a high state of accuracy. Many pitfalls have been recognised and can now with care be avoided, so that an estimation carried out by workers in one country can be fairly constantly assured of corresponding with a test of the same foodstuffs by workers in another country, using modifications peculiar to themselves.

At the same time, so many and varied are the precautions necessary to ensure a constant result, and in spite of all precautions, so liable are the animals used to exhibit individual variations, that many workers, especially in England, consider that chemical tests would be infinitely preferable if they could be instituted. As an example of the wide range of error possible in the biological estimation may be quoted Schmidt-Nielsen's statement (1930) that small doses of vitamin A (ten or even one hundred times less than the usual dosage) give better growth than larger doses. Since the same results could be obtained by greatly varying quantities of fish liver oils it is obvious that their vitamin A content might be quite wrongly estimated.

In the case of vitamin A the chemical tests depending on colour reactions have been widely tested and modified, and their specificity, at any rate for material rich in vitamin A, is established. The elimination of sources of error which might lead to inaccurate values of potency will be further discussed under the heading of vitamin A, as will tests, other than biological, for the other vitamins, under the heading of the individual vitamin. The biological estimation of vitamin B₁ has been found to require considerable modification since the separation of vitamin B into two factors. The difficulty has apparently now been removed by experiments, notably those of Chick and Roscoe (1929), which have provided a basal diet and a source of vitamin B₂ as nearly as possible free from vitamin B₁. The question of the suitability of the rat as the test animal for the antineuritic vitamin is still not entirely settled.

Kinnerly, Peters, and Reader (1928) consider that "it is not yet absolutely certain that rat tests can differentiate the curative factor in the vitamin B complex."

In estimating vitamin B₂ by the rat growth method, the chief difficulty encountered was again the provision of a basal diet and a source of vitamin B₁ free from traces of vitamin B₂. The discovery of Chick and Roscoe that casein must receive very thorough purification to free it from its vitamin B₂ content, and the utilisation of either Peters' antineuritic concentrate or a tiki-tiki extract as the vitamin B₁ source, have obviated this difficulty, and the assay of vitamin B₂ is now satisfactorily established.

Vitamin C, generally estimated from 1922 to 1926 by Sherman's method of producing scurvy symptoms by a diet devoid of vitamin C, is now more sensitively measured by Höjer's tooth method. The latter method has the advantages of fixing accurately the minimum protective dose, and is more speedy than the Sherman method. It will be an additional advantage if Eddy's contention that the ratio of sensitivity of the two tests is that of 1 : 2 is accepted, that the antiscorbutic values already established by Sherman's method will only need multiplying by two in order to bring them up to the accurate values determined by Höjer's test.

The standard of vitamin D, as recommended by the Medical Research Council, is obtained

by the biological method, using either the "line test," the X-ray method, or chemical analysis of the bones. The "line test" is used as the basis of the standardisation of irradiation by the Pharmaceutical Society of Great Britain, together with the Diet 2965 of Steenbock and Black, which is comparatively cheap and easy to prepare, while shortening the period of development of rickets, and minimising the risk of ophthalmia and respiratory infection from too high a vitamin A deficiency.

The method suggested by Scheunert and Schieblich (1929) of taking the unit of anti-rachitic potency as the minimum amount of vitamin D which will, under certain fixed conditions, definitely protect rats from rickets, is also advocated by Hess (1931). The latter suggests a "rat unit," based on the amount of ergosterol necessary to prevent rickets in a rat, rather than comparison with cod-liver oil.

The quantitative estimation of vitamin E is laborious and time consuming, and has the additional difficulty of allowing for the destruction of vitamin E by the oxidising action of other constituents. In the opinion of Evans and Burr, curative tests are more reliable than preventive. The test, therefore, becomes a double one, resorption of the foetus being first established on the basal ration, and then normal fertility restored by the foodstuff to be estimated. Wheat germ oil is generally used as the standard for comparison.

VITAMIN A

OCCURRENCE OF VITAMIN A.—Whether the theory that carotin is the precursor of vitamin A is accepted or not, the results of observations on its formation in nature leave no doubt that vitamin A is synthesised by plants, both land and water. The influence of light on this process is generally accepted as augmentative though not essential. In other words, though there is a slight development of vitamin A in etiolated plants, its greatest synthesis takes place under the influence of light—a fact which is supported by the greater vitamin A content of green leaves as compared with white. The ultimate source of vitamin A in cod-liver oil is undoubtedly the small green diatoms of the sea, which form the food of the small organisms known as plankton, which are in turn the food of the larger organisms and small fishes on which the cod feeds.

VITAMIN A AND PLANT PIGMENTS.—The richness in vitamin A of green plant tissue and tissues carrying yellow pigment led to suppositions that vitamin A might be identical with either chlorophyll or the lipochrome pigments. Xanthophyll also was considered as bearing a close relationship. Since chlorophyll can be separated from vitamin A in plant extracts and since neither lipochromes nor xanthophyll are effective as a source of vitamin A, it is regarded as certain that none of these pigments are vitamin A itself, but that there is a close relationship between them. The extended investigations on the relationship between vitamin A and carotin have provided more definite evidence that carotin may be regarded as the precursor of vitamin A, converted into vitamin A in the animal body, and able to function physiologically as vitamin A itself when injected in very small dosage.

CHEMICAL NATURE OF VITAMIN A.—Although fractionation of vitamin A-containing fats has been carried out to a very high degree, it has so far been impossible to consider any of the active fractions as vitamin A in a state of isolation.

Takahashi's "biosterin," at first claimed to be vitamin A, was shown by Drummond and co-workers to be merely a mixture of highly unsaturated oils, as were Drummond's own more concentrated end-products. According to Drummond and Baker, as recently as 1929, the active principle in the most highly refined concentrates of liver oils is present in such small amounts that attempts to isolate it by ordinary chemical methods are of little use. Their results, however, point to the probability that vitamin A is of the nature of an unsaturated alcohol of the "sterol" group. In this connection the recent investigations of Seel (1931)

are of interest. Seel believes on the basis of his experiments with various products of cholesterol, that the vitamin A formed on oxidising cholesterol is of a sterol nature, and that it is a very labile partial oxidation product of cholesterol. Whether vitamin A can be derived both from carotin and from a sterol cannot at present be definitely stated.

SOLUBILITY OF VITAMIN A.—Apparently the solubility of vitamin A in plant tissues is of a different degree from that occurring in animal fats. In animal tissues the vitamin A dissolves readily in ether, but in plant tissues the results have been discordant, Osborne and Mendel stating that an ether extract of spinach was rich in vitamin A, while McCollum and Pitz asserted that "ether extraction of plant tissue does not remove the substance essential for growth which is contained in butter fat." An alternative explanation to the hypothesis that the plant solubility of vitamin A is different from that of animal occurrence is suggested by Sherman (1931). He points out that an ether extract may contain substances which are not appreciably soluble in pure ether, but which may be soluble in the mixture of fat and ether resulting from the ether extraction of animal tissue containing fat.

COLOUR REACTIONS FOR VITAMIN A.—In view of the known difficulties in instituting extensive biological tests for the quantity of vitamin A in foodstuffs, the introduction of colour tests, specific and satisfactorily accurate, was welcomed in 1922, and their further modification and development from 1925 onwards appeared to render them even more valuable. The antimony chloride reaction in particular was found both sensitive and convenient, and the validity of the method, as tested under the auspices of the League of Nations, was considered proved for cod-liver oil at any rate.

More recent criticisms, however, have thrown doubt upon its value for the quantitative estimation of vitamin A in other foodstuffs and even for the comparative potency of various oils.

The addition of spectroscopic data has decreased the margin of error, but the results of Drummond and Morton, who consider that colour reactions characterised by absorption at $610\text{ }\mu\mu$ are specific for vitamin A, are not in entire agreement with those of other workers, such as Jones and co-workers (1929), Steudel (1929), and Hawk (1929). It seems probable that before the colour reactions are universally accepted as a standard test for vitamin A, a standard technique, excluding all the known sources of error, will have to be devised.

STABILITY OF VITAMIN A.—There are two outstanding points in the question of the susceptibility of vitamin A to destruction. The first is that oxidation is a far more potent destructive agent than heat—a fact which explains many of the early discrepant observations on the resistance of vitamin A in animal fats to relatively high temperatures. Osborne and Mendel's statement (1920), for instance, that butter fat treated with steam, and heated at 90°C . for 15 hours, had not lost its vitamin A activity, was at variance with Steenbock's conclusion (1918) that the vitamin A in butter was comparatively easily destroyed by heat. The presence or absence of oxygen during the heating process has been found to be the determining factor in the vitamin destruction. The second outstanding point is the difference in stability of vitamin A according to its source, animal or vegetable. Apparently the form in which vitamin A exists in plant tissues is one not so readily influenced by oxidation as in animal tissues.

PHYSIOLOGICAL EFFECTS OF VITAMIN A DEFICIENCY.—Of the many aspects of vitamin A deficiency now known and extensively studied, its "anti-infective" action is the one on which attention is at the moment chiefly concentrated. In the early days of vitamin research its growth-promoting property was chiefly emphasised. The cessation of growth which occurs on a diet deficient in vitamin A is undoubtedly a specific effect of vitamin A deficiency, but it has been shown that it is partly dependent upon the stores of vitamin A in the body, and also upon the amount of vitamin D present in the diet. In the adult animal, where the

growth requirement of vitamin A is not so great, a more specific effect of the avitaminosis is the occurrence of xerophthalmia. Until very recently this condition was regarded as so completely specific that vitamin A was called the "anti-xerophthalmic vitamin."

Since the development of two other aspects of the consequences of vitamin A deficiency, its influence on resistance to infection, and its function in maintaining the integrity of epithelial tissues, a broader view of the incidence of eye lesions has been taken by some workers. Instances of this view may be quoted from the observations of McCarrison (1931) and Wright (1931).

The former lays emphasis upon the fact that the initial factor in the invasion of any mucous membrane is the weakening of its structural integrity, vitamin A deficiency being a prominent but not necessarily the sole factor in this weakening, vitamin B also playing a part. The latter also points out that the primary pathological process in the keratomalacia deficiency-complex is one of degeneration and wasting of epithelial surfaces—an epithelial metamorphosis which has been very clearly described by Wolbach and Howe. He agrees with McCarrison that other vitamins may be concerned in the deficiency-complex, and refers to the fact that crude cod-liver oil, containing also vitamins B, C, and D, has a better curative effect than a concentrate of vitamin A alone.

That vitamin A is the principal agent in the initial deficiency, however, is acknowledged by both these workers, and is supported by Pillat's statement that his studies on keratomalacia have convinced him that an increase in the saprophytic bacteria of the cornea, with evidence of epithelial degeneration, is one of the earliest symptoms of vitamin A deficiency in man. Pillat regards the process as primarily degenerative rather than inflammatory. The epithelial changes in vitamin deficiency are not confined to the tissues of the eye. All the mucous membranes, the endocrine glands, the salivary and lymph glands, share in the typical keratinisation, atrophy, and metaplasia of the epithelial cells. The work of Fujimaki (1927) on the occurrence of epithelial hypertrophy in the cardiac end of the stomach is important, especially when correlated with the results of Erdmann and Haagen (1927, 1928) on spontaneous tumour formation in vitamin A deficiency.

Fujimaki believes that the epithelial changes described by him as possibly pre-carcinomatous are related to a disorder of fat metabolism, while Busch considers that the tumours found by Erdmann and Haagen are the response of tissues, injured by some unknown alteration of cell metabolism, to an endogenous irritant produced by the dietary deficiency.

The decreased resistance to infection shown by animals on a vitamin A-deficient diet is so marked that it was but a short step from its observation to the hope that administration of vitamin A would cure infections once established. In this sense, however, it cannot be said that vitamin A has been proved to be strictly an "anti-infective" vitamin. Mellanby, who has greatly extended the observations on this aspect of vitamin A deficiency, remarks that while the experimental results on animals are so clear that it might be expected that the clinical extension to man would also be easy to understand or deduce, this has not as yet been the case. The results of vitamin A therapy in established infections have so far not been "dramatic." At the same time, the value of vitamin A in preventing the incidence of infection is indisputable. The progressive loss of the protective power of the body-surfaces, particularly in the gastro-intestinal and respiratory tracts, undoubtedly predisposes to their invasion by bacteria. The maintenance of this protective mechanism by a diet rich in vitamin A has been amply proved by the animal experiments of McCarrison and of Green and Mellanby. An interesting and suggestive parallel to morbidity in man is drawn by Price (1931) who has pointed out that the incidence of deaths from pneumonia rises in a curve whose phase is inverse to that of the vitamin values of foods consumed by communities. In other words, when the vitamin level of dairy produce is low, as in the winter, the number

of deaths from pneumonia is high. While the administration of vitamin A during pneumonia has not been markedly successful, its administration during periods of low "vitamin tides" may be found to decrease its incidence and mortality. Another instance of infection superimposed upon epithelial tissue already injured by vitamin A deficiency is suggested by Mrs. Mellanby's work on pyorrhœa alveolaris. Whether these results in animals will prove more applicable to man than other aspects of the question of infection remains to be seen.

A final complexity is added to the already difficult question of the "anti-infectivity" of vitamin A by the recent experiments of Topley, Greenwood, and Wilson (1931), their results proving so discordant with those of Webster and Pritchett (1924, 1927). The latter workers dealt with the influence of diet, particularly the inclusion of vitamin A, on resistance to the specific infection of *B. aertrycke*. The former workers concentrated on the influence of epidemic spread of *B. aertrycke* infection, and found that the consumption of vitamin A resulted in no clear difference in susceptibility. They themselves suggest that the normal control diet they employed may have been more favourable than the normal diet employed by Webster and Pritchett. The only conclusion that can be reached is that the relation of diet to infection may be a far more complex question than the mere inclusion of any specific substance in a diet unsatisfactory in other respects.

VITAMIN A IN FOODSTUFFS.—While animal oils, fats, and milk are usually looked upon as the chief sources of supply of vitamin A, certain green plants, particularly spinach, contain comparatively large amounts of it. In all those plants whose leaves show varying amounts of greenness, it is significant that the greenest leaves contain the largest quantities of vitamin A. This fact has been strongly emphasised by Crist and Dye (1931) who suggest that future extensions of knowledge of vitamin A will depend largely upon discoveries in connection with chlorophyll and its functions. With regard to the presence of vitamin A in carrots it is interesting to note that, tested by their potency in promoting rat growth, they are stated by Sherman to be a good source of vitamin A. According to Moore (1931), however, carrots contain no vitamin A as such, but can lead to its production within the animal body by virtue of their content of carotin-bearing substances, hitherto considered good sources of vitamin A.

The vitamin A content of milk is very important in view of the fact that the young of the animal kingdom depend upon it entirely for not only their food but their vitamin supply during early life. Milk and the concentration of its fatty constituents, butter, derive their vitamin supply from plant life, and are of a variable vitamin A content according to the varying activity of plant growth. It is obvious that the vitamin A reservoir of mankind is to a very large extent drawn from dairy products. The production and distribution of these products then takes on an aspect of great importance. The vitamin A value of butter has been disputed, some authorities, including Rosenheim and Webster (1927), regarding it as much inferior to cod-liver oil, while Morton and Heilbron (1930) find it about six times as rich. In any case, the butter from the milk of cows on a summer pasture diet has been found to be richer in vitamin A than that from cows stall-fed on artificial foods. It is fortunate that storage of butter under conditions which do not produce rancidity apparently has no effect upon its vitamin A content—a fact which makes it possible to utilise all the year round the butter richest in vitamin A.

VITAMIN D

SOURCE OF VITAMIN D.—Since the discovery that many foods, devoid of vitamin D as judged by their inability to prevent rickets, are potentially antirachitic, that is, may become vitamin D carriers when exposed to ultra-violet irradiation, the possible sources of vitamin D have been realised to be more extensive than was formerly believed. The source of the

vitamin D in cod-liver oil, one of the richest natural antirachitic products known, has been subject to considerable discussion. The discovery of Leigh Clare (1927), that the marine diatoms believed to be the ultimate origin of the cod's supply of vitamin D were devoid of antirachitic power, cast a doubt upon this supposition. Bills still inclines to the belief that vitamin D can be synthesised in the animal body. Sherman (1931) mentions two other alternatives to this view—either the pro-vitamin may be activated by organic enzymes within the tissues, or the longer waves of ultra-violet light may be productive of vitamin D for fish, while Drummond and Hilditch still incline to the view that the diatoms contain very small amounts of the antirachitic factor, whose cumulative presence in the food provides a sufficient amount to render the cod-liver oil antirachitic.

PRO-VITAMIN D.—That ergosterol is at any rate the most important parent substance of vitamin D is proved beyond doubt. Whether it is absolutely specific in this respect is not at the moment completely agreed. Rosenheim and Webster, basing their belief upon the typical molecular structure of ergosterol, and its specific spectroscopic reactions, believe that no other substance can be regarded as the actual pro-vitamin. Kon, Daniels, and Steenbock (1928) have also come to the same conclusion on the basis that very high amounts of radiant energy were insufficient to activate cholesterol purified by very drastic methods (believed by Bills to be insufficient to destroy their activability).

Others observers, however, including Bills, Honeywell, and McNair (1928), and Koch and co-workers (1929), incline to believe that either cholesterol itself is capable of acting as the pro-vitamin, or that various isomeric forms of certain sterols may attain "pro-vitamin D activity."

MECHANISM OF FORMATION OF VITAMIN D.—Apparently oxidation plays no part in the formation of vitamin D, though it may be involved in its destruction. The temperature co-efficient of the reaction must be very low, for Bills and Brickwedde report that irradiation at the temperature of liquid oxygen produces very little antirachitic activity.

The supposition most generally favoured is that the reaction is a photo-chemical intramolecular phenomenon, involving either a change in position of the double bonds inside the ergosterol molecule, or a "stereo-change" in the alcohol group of the molecule. Bourdillon and co-workers believe that the initial effect of long wave irradiation on ergosterol is to produce at least two substances, only one of which is vitamin D, while Rosenheim and Adam suggest that vitamin D is either a ketone, or one of the ergosterol derivatives produced as by-products during the ketone formation.

ABSORPTION OF VITAMIN D FROM THE SKIN.—Rekling's view that vitamin D is not produced actually by ultra-violet activation in the skin itself but by absorption of activated secretion through the mouth has not been generally accepted. From the facts that "irradiated cholesterol can be absorbed from a small area of undamaged skin in sufficient amounts to supply the needs of the animal" (Hume, Lucas, and Smith (1927)), that human skin contains some 13 to 24 per cent. of cholesterol, and that the longer wave-lengths show a definite penetration of the epidermis, it is believed that the skin may be regarded as an epidermal organ which reacts to particular light waves, acting thus as a potential carrier of vitamin D.

METABOLISM OF ERGOSTEROL.—The fate of ergosterol in the body has not been extensively investigated, but Beumer's (1927) investigations seem to have established the fact that less than half of the ergosterol ingested is excreted in the faeces, and that, unlike cholesterol, it is not excreted in the bile. The portion that remains in the body must be either stored, or resolved into other compounds—Beumer inclines to the latter theory. If it is stored, the place of storage has not been definitely determined, but the observations of several workers that blood, and the sterols of blood, have marked antirachitic power suggest that the red

corpuscles may possibly function in this capacity. Liver, lung, and muscle tissue have also been suggested by Steenbock and Black (1924), and brain, liver, adrenals, and kidneys by Page (1931), as body reservoirs for vitamin D.

PHYSICO-CHEMICAL PROPERTIES OF VITAMIN D—Stability.—Vitamin D is on the whole a more stable vitamin than vitamin A, especially with regard to oxidation. It is also stable to saponification and hydrogenation, but is destroyed by bromination, and forms an insoluble, inactivable, additive product with digitonin.

Vitamin D is sensitive to varying conditions of irradiation. After prolonged irradiation the antirachitic potency of ergosterol decreases, with a simultaneous disappearance of the band at $247\ \mu\mu$. The shorter ultra-violet rays (beyond $260\ \mu\mu$) are also stated by Rosenheim and Webster to be destructive to vitamin D, and they go so far as to suggest that, in order to secure the maximum potency, light filters, cutting off the shorter rays, should be used.

Colour Tests for Vitamin D.—Of the several tests instituted to distinguish vitamin D by specific colour reactions, the absolute specificity of none is definitely established, though Rosenheim's trichloroacetic acid reaction has been perhaps the most used.

Spectroscopic Nature of Pro-Vitamin D.—The discovery that ergosterol possessed a characteristic absorption spectrum in the ultra-violet region, which changes in character during the process of irradiation, has led to much discussion as to what is the true absorption band of vitamin D. Heilbron (1927) believed the new band at $247\ \mu\mu$, formed after about 10 minutes' irradiation, to be characteristic of vitamin D itself, but further researches on the antirachitic potency of the substances formed during different periods of irradiation, have led to the conclusion that the band at $247\ \mu\mu$ cannot be considered specific for vitamin D. With longer irradiation this band can be formed again, the product then being practically non-rachitic. Bourdillon and Webster have now abandoned the supposition which they held in 1929 that the first of the substances produced in succession by irradiation, with maximum absorption at $280\ \mu\mu$, was vitamin D. Their later experiments (1930) have shown that substances with high absorption at $280\ \mu\mu$ may be of very low antirachitic potency, while, *vice versa*, those with low absorption at $280\ \mu\mu$ may be very highly antirachitic.

Thus, at the moment, vitamin D cannot be said to have a specifically characteristic absorption band.

The Antirachitic Wave-Length.—Although it is generally recognised that the portion of the spectral region most favourable to the production of vitamin D is that which lies between $260\ \mu\mu$ and $300\ \mu\mu$, it is no longer considered certain that wave-lengths shorter than $260\ \mu\mu$ are devoid of antirachitic activity. Apparently, however, the short wave irradiation (about $254\ \mu\mu$) is a more complex reaction than the long wave ($275\ \mu\mu$ and longer). Reerink and van Wyk believe that short wave irradiation produces two substances, one of which is vitamin D, but is quickly destroyed on further irradiation. Long wave irradiation, on the other hand, produces only one substance, which is probably vitamin D itself. This latter statement is not, however, confirmed by the most recent observations of Bourdillon and co-workers. They suggest that long wave irradiation also produces at least two substances, only one of which is vitamin D, and that some product of long wave irradiation may be acted upon by subsequent short wave-lengths to form vitamin D. The whole subject is still full of complexities, and Bunker and Harris's suggestion (1930) that "there is still a possibility that the future will show a much wider interpretation of the 'initial band' of rickets therapy" may well be borne in mind.

PHYSIOLOGICAL ASPECTS OF VITAMIN D DEFICIENCY—Bone Lesions in Rickets.—The lesions considered characteristic of rickets are produced with comparative ease in puppies and young rats as well as in other animals. These animals show some variation in the actual histological lesions produced, especially according to variations in not only the vitamin D

content of their diet but also its proportions of calcium and phosphorus. It has, however, been found possible to adjust the diet so that typical rickets is produced, and also so that the deciding factor is the presence or absence of the antirachitic vitamin.

HEALING OF BONE IN RICKETS—The Line Test.—The sensitivity of this test has led to its widespread adoption for the quantitative determination of vitamin D. Positive healing takes place on feeding 0.00002 mgrm. of irradiated ergosterol daily to rats made rachitic with Steenbock's 2965 diet. This diet, which has practically the same calcium-phosphorus relation as McCollum's original rickets-producing diets, is regarded as the most satisfactory for preparing animals for the "line test."

Metabolism in Rickets.—Although the bone lesions constitute the chief clinical sign of rickets, the disturbance of the calcium and phosphorus balance in the blood is the deciding factor in their occurrence. Whether the amount of calcium, or the amount of phosphorus, or the product of the two in the blood is the criterion of rickets is even now in dispute. The recent contention between Compère (1930), attributing the greater importance to phosphorus, and Hess (1931), refuting this contention, may be taken as representative of the two schools of thought.

Although Hess acknowledges that the administration of phosphorus undoubtedly has a calcifying action, he insists that a "phosphorus band" can appear in the metaphyses and long bones without the slightest calcifying effect on the epiphyseal cartilage, and that phosphorus has no field in the prophylaxis of rickets because it has no action on the specific disorder of cartilage and bone which characterises rickets.

Mechanism of the Deposition of Calcium Phosphate.—On this subject again no one of the different theories advanced can be said to be proved, but, in view of the known importance of the balance of calcium and phosphorus in the serum, Robison's picture of calcium and phosphorus ionisation, as influenced by bone and cartilage enzyme activity, is extremely interesting. It accords well also with Shipley's observations on calcification *in vitro*, and it is not at variance with Hess's suggestion that "a local disturbance at the epiphyses may prevent the anchorage of calcium and phosphorus in the cartilage of the bones."

Part played in Ossification by Vitamin D.—That vitamin D is a "stabiliser of calcium and phosphorus" (Hess (1931)) is universally admitted, but its exact mode of action is not completely agreed upon. Ultimately, no doubt, like all vitamin action, it rests upon a basis of cell metabolism and cell structure, and if Drummond's suggestion that the essential function of vitamin D is to produce an increased permeability of the intestinal wall is correct, most of the other theories advanced fall easily into place. If the cell membrane is so altered by vitamin D action that the absorption of calcium and phosphorus is determined, and if, as Telfer, Yoder, Bergheim, and others assert, this absorption is also dependent on the acidity of the medium, the function of vitamin D resolves itself into the double one of preserving a normal intestinal pH and at the same time maintaining the integrity of intracellular action. Bond's hypothesis also rests upon the alterable permeability of cell membranes, in this case of bone cells, and if this action of vitamin D is accepted, together with Robison's theory of enzyme activity, the intimate association between vitamins, organic function, and mineral metabolism is once more strongly emphasised.

Vitamin D and the Parathyroids.—The difference between these two blood-calcium-raising agents has not been elucidated, though the results of Greenwald and Gross, and of Hess, Weinstock, and Rivkin show that there is some connection. Whether the relationship is one of stimulation of the parathyroids by vitamin D, so that the calcium-dissolving power of the plasma is increased, is difficult to state definitely, but apparently vitamin D does exert an auxiliary effect on the raising of the level of the blood calcium by the parathyroid hormone.

Mirvish, correlating the anticalcifying effect of oatmeal with that of ovarian extracts,

even goes so far as to suggest that rickets may prove to be more a manifestation of hypoparathyroidism than a true vitamin deficiency.

The Rachitogenic Factor in Diet.—Mellanby's views on the presence of a factor in cereals, particularly oatmeal, which can produce rickets in animals, has suggested a new aspect of the deficiency theory of the aetiology of rickets. If the action of vitamin D is that of an 'antitoxin,' neutralising the toxic effect of this substance, the conception of the complicated metabolic disturbance produced by vitamin D deficiency will need revision.

From a dietetic point of view the acceptance of either theory makes little difference. As Mellanby points out, whether vitamin D is administered in the diet, or is produced by activation of the ergosterol present in the skin, its presence in sufficient quantity will prevent the noxious effects of the cereal factor.

The Prevention and Treatment of Rickets.—Of the several methods now available for the prevention and treatment of rickets the choice is more or less one of individual preference and convenience, for all have proved satisfactory in the hands of different observers. In France, sunlight clinics have proved very successful in the prophylaxis of rickets, especially in premature infants, notably susceptible to its development. The regular administration of artificial ultra-violet rays is also very satisfactory in preventing rickets, and in Vienna especially has shown itself of great value in curing the disease. The optimum exposure, however, must be carefully standardised, and ultra-violet therapy has the disadvantage of the necessity of special clinics for its satisfactory administration.

When the choice between cod-liver oil and irradiated ergosterol has to be made, there is on the one hand the disadvantage of the fact that the large doses of cod-liver oil necessary may interfere with the digestive assimilation of the child, while, on the other hand, irradiated ergosterol, or equivalents such as viosterol, are known to produce toxic effects if an overdose is given. Recent researches and careful standardisation have to a great extent overcome this difficulty, and American workers are in general firmly convinced of the superior efficacy of irradiated ergosterol over any other form of rickets therapy. Hess, however (1931), while strongly advocating the use of viosterol, and minimising the risk of hypercalcaemia from overdosage, points out that the relationship between the amount of cod-liver oil and ergosterol necessary to prevent rickets is 1 : 3. He suggests that the explanation may lie in some additional unknown antirachitic factor possibly present in cod-liver oil.

The administration of irradiated foodstuffs has received great impetus since Steenbock and Black's discovery that a very wide range of foods could be made antirachitic by exposure to ultra-violet rays. The exponents of this method show great enthusiasm for it, claiming that the disadvantages of toxicity from overdosage are almost entirely eliminated, and that good effects on the general health are additional benefits of its employment. Irradiated milk and irradiated yeast are two substances which have received specially favourable reports. In the case of milk it has been found necessary to elaborate methods which would overcome the double disadvantage of the production of a disagreeable taste and flavour, and the possibility of destruction of its vitamin A potency.

Effects of Large Doses of Irradiated Ergosterol.—While the questions of the degree of toxicity of overdosage of irradiated ergosterol, and of its effect being due to a pure hyper-vitaminosis D or to associated toxic substances are still in dispute, there is practically universal agreement that overdosage does produce harmful effects. Pathological changes, varying from abnormal deposition of calcium in the various organs to symptoms of acute intoxication and death, have been reported by numerous observers.

Since the standardisation of dosage has been carried out, however, it is probable that overdosage of vitamin D itself from administration of irradiated ergosterol has become very improbable. Hess, in particular, believes that the dosage necessary to produce toxic effects

is so large that the danger is practically negligible. Whether the toxicity is due to vitamin D itself or to associated substances present either as impurities or produced during irradiation cannot definitely be stated. Harris and Moore believe that it is the vitamin D produced by optimum irradiation which causes a specific hypervitaminosis, since both over-irradiated and non-irradiated ergosterol are non-toxic, while Hoyle believes that an unknown toxic substance is also concerned.

In view of the recent developments on the mechanism of production of vitamin D during irradiation, and the suggestion of Bourdillon and others that at least two substances are formed during the process, it would be interesting to know the relative toxicity of these products, if such tests could be carried out.

Mechanism of Hypervitaminosis D.—The exact mode of action of the production of lesions by overdosage of vitamin D is not known further than that the essential change is a profound disturbance of the calcium and phosphorus level of the blood. There is undoubtedly at one stage of the process a hypercalcaemia, and also a hyperphosphataemia; the latter, however, Hess believes to have no other significance than that of a stabilising action on the part of the body. The source of the increased calcium in the blood is believed to be not only the food ingested, with increased absorption through the intestinal wall, but also the bones themselves. Brown and Shohl believe that an actual demineralisation of the skeleton takes place, a mobilisation of the calcium reserves, with their subsequent deposition in abnormal positions. This theory is supported by the work of Bauer, Aub, and Allbright, and of Greenwald and Gross, while Harris and Innes (1931) state that the relative importance of these two sources of supply is determined by the available calcium in the diet and by the degree of vitamin D excess.

Other Therapeutic Effects of Vitamin D.—According to the reports of numerous observers, referred to in the text, vitamin D would seem to have a wide field of efficacy as a therapeutic agent. Many of these effects, however, can scarcely as yet be regarded as more than suggestions with some foundation of truth. Such disorders as ozæna, dementia præcox, pernicious anæmia, etc., cannot be looked upon as specifically amenable to vitamin D therapy until a much greater number of cases have been tested. There are, however, four aspects of treatment by vitamin D which are of practical importance—osteomalacia, calcification of teeth, antibacterial resistance, and consolidation of fractures.

OSTEOMALACIA.—If, as Hess states, the underlying pathological lesions in rickets and osteomalacia are the same, it would seem reasonable to conclude that treatment which has proved eminently satisfactory for the one disease should do so for the other. To a certain extent the conclusion is apparently justified, especially in vitamin D therapy by irradiation (particularly sunlight) and by cod-liver oil. In India, osteomalacia is largely a disease of darkness, ameliorated by exposure to light, while a simultaneous correction of a diet deficient not only in vitamins but in calcium has been shown to have good results. Cure of the bone lesions, producing a deformity more permanent in adult than in young growing bone, is not so rapid or so striking as in rickets.

CALCIFICATION OF TEETH.—Mrs. Mellanby's work on the pathological processes in the development of caries in teeth is of the greatest importance from the point of view of infant feeding. If the actual formation of new teeth is a normal process, resulting in the development of strong healthy teeth, resistant to attrition and bacterial invasion, the most serious aspect of the problem of dental caries in later life disappears. "A perfect diet in the developmental period either prevents or greatly inhibits the production of periodontal disease at a later stage, even if the diet is subsequently deficient in vitamins A and D." Another interesting point which emerges from the later researches is the connection between vitamins A and D in the tooth-forming process. If vitamin D guards the alveolar bone from maldevelopment

and subsequent injury, vitamin A guards the soft periodontal tissues. The importance of further research on this subject, from the point of view of the wide prevalence of pyorrhœa, can scarcely be overestimated.

ANTIBACTERIAL RESISTANCE.—Though vitamin D has not such a definitely “anti-infective” action as vitamin A, reduction of vitamin D in the diet has apparently the effect of lowering the resistance to bacterial invasion. The effect can scarcely be said to be specific, however. Especially in tuberculosis the amelioration of symptoms by administration of cod-liver oil cannot be regarded as entirely due to the antirachitic factor contained in it. Besides the presence of vitamin A, there is the possibility, receiving more and more attention recently, that cod-liver oil, even in its curative effect upon rickets, is valuable for some unknown factor besides vitamin D.

CONSOLIDATION OF FRACTURES AND DECALCIFICATIONS.—Apparently the optimum effect of vitamin D in promoting normal calcification depends upon the correct dosage. While very large doses may produce excessive calcification, it does not follow that this calcification will produce normal healed bones. Morelle suggests a dosage of three to four times the therapeutic dose for rickets, and with this dosage has had good results.

Vitamin D in Foodstuffs.—The richest sources of vitamin D are fish oils, egg-yolk, butter, milk, and some green vegetables, but except in the case of cod-liver oil and some other fish oils, none of these foodstuffs can compare in vitamin D with vitamin A content. The occurrence of vitamin D in fish oils varies considerably with different species and even within one species.

Milk and butter are now known to be less rich in vitamin D than was at first supposed. The question as to whether the vitamin D content is greatly influenced by either the diet of the cow or its exposure to sunlight or ultra-violet irradiation is not quite clear. Apparently fresh green food and sunshine are both effective in improving the antirachitic quality of the milk but not to such an extent as to exalt the importance of one factor above the other.

VITAMIN E

It is unnecessary to discuss at great length the experimental evidence for the existence of the antisterility vitamin. The careful and conclusive investigations of Evans and Burr, supplemented by the additional evidence of other workers such as Sure and Mattill, have proved beyond doubt that not only does a vitamin exist with a specific effect upon reproduction, but that the disturbance of function produced by its deficiency is of itself specific in nature. The failure to reproduce normally on a vitamin E-deficient diet differs entirely in character from the failure produced by vitamin A or B deficiency in that normal conception can take place while the production of living young is absolutely prevented. Resorption of the foetus is the pathognomonic sign of vitamin E deficiency in the female, and the difference of effect of the deficiency upon the female and the male is another very characteristic feature. In the female the resultant sterility is always curable, because the generative tissues themselves remain unimpaired, while in the male a more or less complete degeneration of the testicular tissue ultimately takes place.

The physical and chemical properties of vitamin E have shown it to be a fat-soluble substance, with a range of solubility greater than that of ordinary fats. It is also a remarkably stable vitamin, resisting many of the chemical reactions towards destruction. It has, however, one reaction towards destruction that has led to some discrepancy in the results of testing for its presence in food materials. This is its destruction by some factor present in some fats and oils. Evans and Burr at first considered this effect to be due to the mechanical removal of vitamin E by excess of fat in the ration, while Mattill believed it due to the development of rancidity. Evans and Burr now agree with Mattill as to the destructive

effect of rancidity but consider the effective factor in destruction an "antivitamin" substance, not necessarily specific for vitamin E.

CONCENTRATION OF VITAMIN E.—A complicated fractionation of wheat germ oil has resulted in the isolation of a yellow viscous oil which cures sterility in the female in a single dose of 5 mgrms. while the administration of 0.3 mgrm. daily to the male results in maintenance of normal reproductive function. As in the case of vitamin A, however, this fraction is still considered impure, and not to consist of the vitamin itself.

VITAMIN E IN FOODSTUFFS.—The distribution of vitamin E differs from that of the other fat-soluble vitamins in that its richest occurrence is in vegetable rather than animal tissue. Wheat embryo, lettuce leaves, and alfalfa all yield extraordinarily potent extracts, while cod-liver oil, milk, and butter are comparatively poor sources.

THE WATER-SOLUBLE VITAMINS

THE VITAMIN B COMPLEX.—Vitamin B, as formerly understood, no longer exists. At least two factors, with differing chemical and physiological properties, must be considered and discussed under the heading of "vitamin B," while three, if not more, other substances concerned in nutrition and having a more or less similar distribution are now postulated.

VITAMIN B₁—Occurrence.—The water-soluble antineuritic vitamin occurs in a wide variety of natural foodstuffs, being present in connection with the growing capacity of the cells. The chief sources are seeds, eggs, and yeast. In seeds the embryo contains the largest amount, while the endosperm, when the aleurone layer is removed by manufacturing processes, is almost devoid of the vitamin.

Chemical Nature of Vitamin B₁.—Ever since Funk's strenuous attempts to identify the antineuritic vitamin with the class of pyrimidine bases, an enormous amount of work has been expended on trying to establish its chemical identity.

The quinoline and glyoxaline derivatives claimed to be curative of polyneuritis by Sahashi have given negative results under the carefully standardised experiments of Gulland and Peters (1929), while the di-keto piperazines tested by Williams and Eddy (1927–1928) have proved not convincingly positive, and of the numerous other compounds tested none have been found to be completely identifiable. In spite of the successful concentration of vitamin B₁-containing substances into fractions so small that they are curative in a dosage of 0.027 mgrm. per day (Kinnersley and Peters' "torulin") and 0.005 mgrm. per day (Guha and Drummond's wheat germ preparation), no closer agreement than that of Drummond and Peters that the antineuritic vitamin is a basic substance (according to Drummond "of relatively simple composition") has been reached.

Concentration of Vitamin B₁.—Starting from Funk's method (1911) of concentrating the antineuritic vitamin in rice polishings by precipitation with phosphotungstic acid, many other methods have been elaborated, involving very complicated chemical processes and employing other substances, including yeast and wheat germ. The essential steps in concentration are:

(1) **EXTRACTION** from the basic material, generally with alcohol (70 per cent. is the strength most commonly used) with water, acidified with sulphuric acid, or with dilute acetone or glacial acetic acid.

(2) **PURIFICATION**, to remove proteids and lipoids. Osborne and Wakeman's method of removing protein was to coagulate it with boiling water. Funk's method of removing lipoids was repeated extraction with ether.

(3) **PRECIPITATION.**—Funk used phosphotungstic acid, followed by silver nitrate and barium hydroxide, the active substance (which he called "vitamine") being found to some extent in the precipitate but a good deal remaining in the filtrate. The "oryzanine" of

the Japanese workers was obtained by a method closely following Funk's procedure, and other workers used various other similar precipitating reagents. The disadvantage with all these methods was that much of the active substance was lost during the fractionation of the precipitate.

In 1916 Seidell made the discovery that a special form of fuller's earth effectively removed the active substance from yeast by adsorption. The activated fuller's earth could now be washed, further fractionated, and dried in a vacuum, giving a final product highly potent against polyneuritis in pigeons. Further modifications of his original procedure have been carried out by Seidell, chiefly with the object of liberating the active substance from its combination with fuller's earth without unnecessary loss. Another modification of the adsorption method was introduced by Levene and van der Hoeven (1924). They used Osborne and Wakeman's method of preparing the yeast for final concentration, and used silica gel as their principal adsorbing agent. It was later found (after the separation of vitamin B into its two components) that silica gel adsorbed both vitamin B₁ and B₂, but preferentially the heat labile factor. From the knowledge of this fact, Levene and van der Hoeven have devised a method for preparing a fraction free from vitamin B₂ by deaminising the starting material with nitrous acid.

Jansen and Donath's method also depends on adsorption on a variety of fuller's earth (acid clay) and subsequent extractions, purifications, and re-purifications of the adsorbed material. Platinic chloride is used as one of the final precipitating reagents, and their final product is a "vitamin hydrochloride"—a very potent antineuritic substance.

A new adsorbent in the form of "norite" (vegetable charcoal) has been used by Peters and Kinnersley, who, by further elaborate purifications and improvements of technique, have prepared a substance which they call "torulin." They have, however, failed to find Jansen and Donath's method for rice-polishings applicable to their yeast product, either, they state, because some peculiarity in yeast extracts prevents its efficacy or because the "anti-beri-beri factor may be distinct from torulin."

Guha and Drummond's concentrate from wheat germ is apparently of higher concentration even than torulin, and it raises the interesting question of the duality of vitamin B₁. The supplementary action of an inactive filtrate from a gold chloride precipitation to the active precipitate seems to show that more than one factor is concerned in the activity of the antineuritic vitamin.

Chemical Properties of Vitamin B₁.—Although regarded as a water-soluble vitamin, vitamin B₁ is soluble to some degree in lipoid solvents such as ether and benzene, especially if it is extracted first by alcohol.

Its capacity for dialysing through collodion membranes has given rise to a tentative suggestion as to its possible molecular weight, since substances with similar dialysing properties have molecular weights of about 300–500.

Stability of Vitamin B₁.—The application of the term "heat labile" to vitamin B₁ is somewhat misleading, for though more liable to destruction by heat than vitamin B₂ it is none the less stable to comparatively high temperatures, even 130° C. destroying only 55 per cent.

Oxidation apparently plays little or no part in its destruction, though the observations of Daniels, Giddings, and Jordan (1929) indicate some influence of aeration in the case of heated milk. Hydrogen-ion concentration is, however, an important factor; the destruction of vitamin B₁ in tomato juice heated to 100° C. for one hour being, according to Sherman and Burton, about 10 per cent. at pH 5.2 and 90 to 100 per cent. at pH 10.9.

Vitamin B₁ and Metabolism.—The aspect of the relation of vitamin B₁ to metabolism which has given rise to most discussion is its relation to tissue oxidation. The fall of tempera-

ture and the respiratory difficulties occurring during severe vitamin B₁ deficiency gave rise to the idea that vitamin B₁ was specially concerned with the oxidising mechanism of the body tissues and that tissue oxidation was definitely lowered by its absence.

Much evidence was brought forward in support of the "oxidation theory," W. R. Hess maintaining that the administration of yeast would restore the tissue oxidation to its normal level, while Randoin and Fabre and de Mattei based their contention on the diminution of glutathion in various tissues.

Drummond and Marrian, however, by a careful study of the oxygen intake of isolated tissues, and of the gaseous metabolism of rats whose temperature was raised by immersion in a warm bath, finally related the difference in metabolic rate to the fall of temperature produced by starvation rather than to a specific effect of B-avitaminosis. The same is apparently true of the disturbance in carbohydrate metabolism which takes place in the final stages of vitamin B deficiency, though Peters believes that vitamin B₁ is definitely related to the oxidation of lactic acid, and Lepkowsky, Wood, and Evans (1930) that, in animals showing definite signs of beri-beri, there is evidence of interference of vitamin B₁ with intermediary carbohydrate metabolism.

In protein metabolism the relation apparently concerns vitamin B₂ rather than vitamin B₁.

With regard to fat metabolism the observations of Evans and Lepkowsky (1929) are considered to show that vitamin B₁ has a definite effect in "sparing" the body requirements for fat.

Vitamin B₁ Requirements.—Apparently different species of animals have a different optimum intake of vitamin B₁, fowls having a much higher requirement for instance than cats and dogs. During lactation also the maternal requirement of vitamin B₁ is increased by three times that necessary for normal growth, and, further, the nursing young are liable to suffer from vitamin B₁ deficiency even when the mother's vitamin intake is increased, since, except in the case of the cow, she is apparently unable to pass on the whole of the supplementary vitamin into her milk. For this reason Sure has stated that a judicious distribution of vitamin B₁ between the mother and the young has a good effect on infantile mortality. The cow, on the other hand, seems to be able to provide plenty of vitamin B₁ in its milk independently of its intake—a fact which has received an explanation by the supposition that vitamin B₁ is actually formed during bacterial fermentation in the digestive apparatus of the cow.

BERI-BERI—Etiology.—Considering that it was from his observations on beri-beri occurring in prisoners that Eijkman first formulated his conception that some substance in the polishings of rice prevented the disease, it is somewhat extraordinary that the causation of beri-beri should have been so long and so variously discussed. For it was from Eijkman's conception that the whole theory of vitamin B deficiency arose, though Eijkman himself was at that time far from recognising the true nature of the substance which prevented polyneuritis in fowls in the prison yard. Yet, even so recently as this year at a meeting of the "Société de médecine et d'hygiène tropicales," opinions were advanced that beri-beri is not a deficiency disease but rather a "toxic-infection." If this theory is entertained it is very difficult to explain the undoubted experimental production by withholding the anti-neuritic vitamin from the diet of a disease in animals so closely resembling beri-beri in man. The "rice toxicity" theory is more easily reconcilable with the vitamin deficiency theory, for any factor which tends to denude rice of its vitamin B₁ content would make it potentially "toxic" if toxicity is regarded in a negative "deficiency" sense.

Most upholders of the "infective" theory, however, add a rider to their view that beri-beri is due to infection by a specific bacillus. They state that the vitamin deficiency is a predisposing factor in the invasion of the body by the organism concerned. How far beri-

beri in man is a pure deficiency disease it is at present impossible to state. It can only be said that radical changes of diet in accordance with the acceptance of the vitamin deficiency theory have undoubtedly led to its eradication in communities suffering from beri-beri, and that careful investigations into the incidence, symptoms, and cure of what is believed to be the same disease in animals have shown that the substance known as vitamin B₁ is undoubtedly its specific cause.

Clinical Aspects of Beri-beri.—It is interesting to note in connection with the recent emphasis laid on partial vitamin deficiency that a “pre-beri-beri” condition is now recognised. The importance of prophylactic treatment, in order to prevent the appearance of symptoms, which, once established, are not only difficult to cure, but are easily precipitated into frank beri-beri, is obvious.

Pathological Changes in Beri-beri.—While the degeneration of the peripheral nervous system is one of the most constant lesions occurring in beri, it is apparently less extensive and less permanent than it was at first believed. The actual lesion is a swelling, loss of differentiation, and chromatolysis of the nerve endings. Some observers, such as Honda and Bito, locate the lesion in the Schwann cells of the nerve sheath, while Woolland believes that the myelin suffers more than the axis cylinder. In any case, all the lesions disappear with remarkable rapidity on administration of vitamin B₁—a fact which has led to the conclusion that a true nerve degeneration cannot be present. Wenckebach provides a very acceptable explanation by his theory that the essential cause of beri-beri is a “water retention,” which would account for the swelling of the cells and its disappearance when the factor whose absence causes the disturbance of “water balance” is restored. His explanation also accords well with the histopathology of the heart lesions and the oedema of the tissues which are more or less constantly present in beri-beri. He believes that the characteristic enlargement of the right side of the heart is neither a hypertrophy nor a true oedema but a specific “water retention,” as is also the oedema of the superficial tissues. If Peters’ hypothesis is accepted that a third factor present in yeast (which he calls the “anti-oedema” factor) is needed to act in combination with the pure antineuritic factor in order to produce an “anti-beri-beri” vitamin, some of the discrepancies in the results of treating beri-beri with large doses of vitamin B₁ may be explainable, as well as Wenckebach’s “water-retention” theory. McCarrison, on the other hand, lays stress on the connection between the occurrence of oedema and the hypertrophy of the adrenals constantly found in vitamin B₁ deficiency.

Experimental Polyneuritis in Animals.—One of the greatest sources of confusion in the question of whether experimental polyneuritis in animals corresponds to beri-beri in man has been the fact that different degrees of vitamin B₁ deficiency produce a different symptom complex. McCarrison, in 1928, emphasised this difficulty and cleared up much of the confusion when he pointed out that the basal factor in the production of beri-beri columbarium is insufficiency, but not complete lack, of vitamin B₁, while typical avian polyneuritis is produced by an entire absence of the vitamin.

Further Physiological Aspects of Vitamin B₁ Deficiency.—Many workers hold that the partial deficiency of vitamin B₁ is a far more prevalent condition than is generally realised, and that its effects are more important and more productive of disease than that of any other vitamin deficiency. There are others who hold the view that many of the effects noted as due to vitamin B₁ deficiency are in reality due to the chronic starvation which accompanies it. The loss of appetite, loss of weight, and fall of body temperature, which are three of the most characteristic features of vitamin B₁ deficiency, have all been attributed to inanition rather than to a specific effect of vitamin B₁.

If the conception of vitamin B as specially concerned with cell metabolism is accepted, Peters’ view of the mechanism of production of anorexia and fall of temperature provides a

reasonable explanation. He believes that both reactions are protective in nature, an adjustment of the tissues against their disorganisation by vitamin B deficiency.

Lesions in the Alimentary Tract.—Congestion, atrophy, and bacterial invasion have been shown to occur in the intestinal canal, but whether these changes are specific to B-avitaminosis or a manifestation of the underlying cell disturbance produced by any vitamin deficiency, or by inanition, is difficult to determine. Impairment of motor function has been fairly conclusively demonstrated, and evidence has been presented that substances rich in vitamin B have a stimulating effect on the secretory function of the whole tract, but further research is necessary to prove that these effects are entirely specific to vitamin B₁.

Changes in Endocrine Organs.—The most striking change in the endocrine system produced by vitamin B₁ deficiency is undoubtedly the hypertrophy of the adrenals, emphasised particularly by McCarrison. Although a similar hypertrophy occurs in inanition, it is apparent from the work of Marrian (1928) that vitamin B₁ deficiency itself and separately does produce the lesion. What is the exact significance of the hypertrophy cannot be said to be definitely proved. McCarrison believes it to be an important link in the chain of metabolic disturbance which results in the various manifestations of avitaminosis, especially in the genesis of oedema, but his thesis is not universally accepted.

There is a similar lack of agreement as to the many other effects reported as due to vitamin B₁ deficiency, and it would seem that, until more complete investigations have been carried out, the most definite statement that can be made is that minor degrees of vitamin B₁ deficiency lead to loss of appetite with progressive failure in nutrition, with possible digestive disturbance, and secondary disturbances of health dependent upon endocrine insufficiency.

Refection.—The spontaneous recovery of rats from the effects of vitamin B deficiency has been shown to be due to their consumption of faeces in which vitamin B has apparently been produced by bacterial synthesis in the intestinal tract. The phenomenon has been shown to be possible only when the diet contains a large proportion of starch, and Fridericia has postulated the existence of a specific virus for refection, which he has not, however, been able to isolate.

Vitamin B₁ and Malignant Disease.—It cannot be said that vitamin B has any specific effect on the development of malignant tumours. Even if those investigations which claim to show that tumours develop less rapidly on a diet deficient in vitamin B₁ are accepted as incontrovertible, the phenomenon is explicable, as Drummond has pointed out, on non-specific grounds. Drummond suggests that vitamin B₁ is only one of several agents which are quickly exhausted in tissue reserves. When this exhaustion is complete the rate of tumour proliferation decreases, and therefore this decrease will occur in the early stages of either vitamin B₁ or certain protein deficiencies.

Vitamin B₁ in Foodstuffs.—The location of vitamin B₁ in the embryo of cereals, and the removal of the embryo by many milling processes, has doubtless led to a considerable diminution of vitamin B₁ in the modern diet, of which cereals form a large part. A diet consisting largely of white bread and polished rice is definitely productive of a degree of vitamin deficiency sufficient to produce disturbances of health. Since vitamin B₁ has a wide distribution in other foods, the deficiency would not require much consideration if other foods were taken in due proportion, but in times of economic stress, and also when communities are ignorant of the vitamin value of foods in general, a real shortage might be produced. Full knowledge of the danger should, however, prevent such a possibility, and where the whole grain of cereals cannot always be used, it should be possible to incorporate some of the elements removed into the finished manufactured product. Egg-yolk and milk are two other notable sources of vitamin B₁. According to Sherman, egg-yolk contains 50 units per ounce of vitamin B as against milk 9 units. Milk is somewhat variable in its vitamin B₁

content. Cows' milk in particular depends not only upon the diet of the cow, but also upon the bacterial synthesis of vitamin B in the rumen of the cow. Human milk is possibly differently constituted from cows' milk with regard to its content of the water-soluble vitamins B₁ and B₂. Commercially dried milks appear to lose little or none of their vitamin B₁ value, especially when the roller process is used.

VITAMIN B

SOURCE OF VITAMIN B₂.—Although the general distribution in foodstuffs of the two water-soluble vitamins is very similar, the correlation is not exact. Some vegetables and pulses, for instance, are much richer in vitamin B₁ than B₂, while milk is a better source of B₂ than B₁.

CONCENTRATION.—The separation of vitamin B₂ from vitamin B₁, occurring in the same substance, has been attended with some difficulty. The fact that the antineuritic vitamin is the more easily destroyed by heat has made it possible to obtain a fairly rich source of vitamin B₂ from yeast by autoclaving, but some of the growth-promoting vitamin is destroyed in the process.

During the concentration of vitamin B₁, the process of precipitating with lead acetate was found by Chick and Roscoe to remove most of the vitamin B₂. The separation of vitamin B₂ from the lead acetate could now be accomplished by decomposing the precipitate, but the results were not entirely constant.

Another method was devised from the fact that vitamins B₁ and B₂ are differently adsorbed by fuller's earth, so that by filtration and successive fractionations a practically pure source of vitamin B₂ can be obtained. Levene (1930) has concentrated a substance which is sufficient to maintain normal growth of rats in a dosage of 0.0007 gm.

PHYSICO-CHEMICAL PROPERTIES.—The reaction of vitamin B₂ which has been most disputed is its stability to nitrous acid. The most recent observations, Guha (1931) show that it is not destroyed by nitrous acid. With regard to the statement in the text (pp. 295–296), that vitamin B₂ is more completely adsorbed by fuller's earth than vitamin B₁, it may be remarked that Guha merely states that norite adsorbs the factor at pH 4.6 but that it cannot be easily elutriated. Guha also states that vitamin B₂ is probably not a base, an acid, or a peptide, but a neutral substance.

PELLAGRA.—The ætiology of pellagra presents even greater difficulties and uncertainties than that of beri-beri, for pellagra-like symptoms produced in experimental animals are less like pellagra in man than beri-beri columbarium is like beri-beri hominum. The application of experimental results to clinical manifestations becomes therefore more difficult.

The theory of an infective origin of pellagra has been more or less abandoned since Goldberger's extensive investigations into the occurrence of pellagra on vitamin-deficient diets, but it cannot yet be said that pellagra is a specific effect of deficiency of vitamin B₂.

Many workers believe that the disease in man may be a syndrome representing a multiple deficiency not only of vitamin but of protein, while Mellanby's suggestion that the connection between maize and pellagra may be the presence of a protective "vitamin" factor which neutralises the toxic effect of maize has yet to be fully investigated. The discovery of another animal condition similar to pellagra and preventable by the same dietary methods (black tongue in dogs) has introduced a further complication. As Aykroyd has pointed out, diets which produce pellagra in man and experimental black tongue in dogs do not necessarily produce pellagra-like symptoms in rats.

The recent investigations on the influence of vitamin B₂ on protein metabolism must be taken into consideration in this connection, especially in view of Wilson's contention that the "biological value of protein" is the determining factor in the occurrence of pellagra. It

is not impossible that abnormal metabolism of protein in the absence of vitamin B₂ may be a factor of some importance in the multiple deficiency, if such it be, which produces the separate manifestations of "rat pellagra," black tongue, and human pellagra.

The effect of vitamin B₂ deficiency in rats is nevertheless the production of a definite set of symptoms which include lack of growth, skin lesions of a specific character, lesions of the nervous system and alimentary disturbance. The skin and nervous lesions resemble closely those of human pellagra, the degeneration of the nerve cells of the spinal cord being a particularly striking correlation of the two diseases. In experimental black tongue also the resemblance to pellagra is found. It is pointed out by Denton that "the lesions of both appear to have their inception in a degenerative process in an analogous tissue element." Whether the factors concerned in the production of the three conditions are identical it is at present impossible to say.

VITAMIN B₂ IN FOODSTUFFS.—Animal tissues and milk are much richer in vitamin B₂ than B₁, while cereals and most vegetables and fruits are lower. Bananas, however, are stated by Eddy to contain at least three times as much vitamin B₂ as B₁.

VITAMIN C

Although recent investigations have suggested that vitamin C, like vitamin B, may consist of two factors, these factors are probably not to be regarded so much as separate entities, as distinct properties of vitamin C. Attempts by Bezssonoff and Randoin and Lecoq to isolate two separate factors have not been entirely successful, and Zilva does not suggest that the two factors which he calls the "thermolabile factor" and the "reducing agency" respectively are actually preformed in the living cell. Rather does he believe that the presence of the reducing agency is necessary for the cell equilibrium on which depends the stability of the antiscorbutic factor, and that its removal during chemical fractionation means the destruction of its protective action.

SOURCE OF VITAMIN C.—Unlike vitamin B, though apparently potentially present in dry seeds, vitamin C is only available when the living cell is in actual process of germination. The question whether vitamin C is actually activated before visible germination takes place is an interesting one. The experiments of Kucera, Luettmerding, and others on the effect of soaking seeds suggest that the vitamin C preformed in the ripened seed does become activated when the seed is soaked; in other words, that the mature seed contains something which may be regarded as the "resting stage" of the vitamin which becomes fully formed in the process of germination.

CHEMICAL NATURE OF VITAMIN C.—Zilva's experiments resulting in the postulation of the two factors in vitamin C have led to a revised conception of the chemical structure of vitamin C.

The presence of traces of nitrogen, iodine, sulphur, etc., in the purest fractions obtainable, are now believed to co-exist as impurities and not to be part of the molecular structure. At the same time Zilva now believes that the molecule, instead of being approximately the same size as a hexose, may be definitely larger than his earlier studies in dialysis led him to surmise.

CONCENTRATION.—Lemon juice is generally used as the material from which it is attempted to concentrate vitamin C. Zilva's methods or modifications of them have proved very successful in avoiding the destruction of the vitamin by oxidation, and in removing the impurities closely associated with the potent fraction. The removal of the organic acids by powdered chalk, the removal of sugar by fermentation with yeast, and precipitation of the active material by basic lead acetate at pH 7.2, were fundamental principles in the process of concentration upon which other workers have based their methods. Grettie and King (1929) use butyl alcohol to precipitate the yellow colouring and waxy matter of lemon juice, while

Sipple and King (1930) introduce a further modification in the form of absolute ethyl ether to remove inactive material. The results of these latter workers in attempting to use precipitating reagents other than lead acetate suggest that the phenomenon of precipitation of vitamin C by lead at an optimum pH is one of specific adsorption.

STABILITY OF VITAMIN C.—The susceptibility of vitamin C to destruction by oxidation has an important bearing upon the question of its preservation in canning processes. Efforts to prevent this destruction have led to special methods being used by Kohman, Eddy, and co-workers. They believe that heat is not so potent a factor in vitamin C destruction as oxidation, and that if oxygen can be excluded little vitamin C is lost in the process of canning. A vacuum exhaustion and preliminary treatment with brine to exhaust the respiratory oxygen are among the methods now used in modern canning of fruits and vegetables. The effect of heat is of some importance in the question of the vitamin C content of milk subjected to various processes, though Hess believes that the associated conditions are of more importance than the actual degree of heat. If pasteurisation, for instance, is carried out with due regard to the exclusion of oxygen as far as possible, there is apparently little destruction. Time is also an important factor in destruction by heat. Delf (1918) found that 20 minutes' heating at 90° or 100° C. was about equal in destructive effect to 60 minutes at 60° C.

Vitamin C is susceptible to alkalinity at low temperatures—a fact which has been much emphasised in the education of the public with regard to cooking. Preservative agents, necessary to prevent fermentation in fruit and vegetable juices, are unfortunately destructive to vitamin C in proportion to their preservative potency.

VITAMIN C AND METABOLISM.—Vitamin C appears to have no special connection with general metabolism further than that which must be considered in all vitamin action, the maintenance of the normal metabolic activity of the cell.

In the metabolism of iron and calcium, however, it appears to have some special importance.

Mouriquand and Leulier have correlated the occurrence of anæmia in scurvy with a characteristic disturbance of iron metabolism, and conclude that in vitamin C deficiency the reserves of organic iron in the liver become exhausted.

Defective calcification of the bones is one of the signs of scurvy, and though observations on the calcium balance in the blood have had somewhat inconclusive results, the recent experiments by Salter and Aub (1931) seem to indicate a close connection between the utilisation of bone salts and a sufficiency of the antiscorbutic vitamin. These workers have found that in animals receiving a diet devoid of vitamin C no new bone salts were deposited during the various stages of scurvy, while when healing took place under the administration of a source of vitamin C, deposition in the trabecular system as well as in the epiphyseal region was clearly shown. The conclusion that the disturbance in calcification may affect the storage of bone salts in the trabecular tissue is of some importance in view of the fact that administration of vitamin C may help to minimise the drain on calcium salts in times of abnormal stress, such as lactation.

SCURVY.—There are practically no dissenters at present from the view that scurvy is a deficiency disease; the connection between cause and effect has been shown with considerable clearness both in human scurvy and the experimental production of similar symptoms in animals. The surprising statement (1931) by Charcot (*Brit. Med. Journ.*, i. p. 862) that scurvy is not so much due to the lack of fresh foods as to the use of canned meat will no doubt be further explained when further investigations have been carried out. An interesting feature of the clinical occurrence of scurvy is the existence of a prescorbutic condition, due to a deficiency of the antiscorbutic vitamin. Like the condition described as prescorbutic condition may be easily precipitated into the recognisable disease, and in the

case of scurvy it appears possible that the determining factor may be some superimposed infection. A "latent" form of infantile scurvy also appears merely as a state of malnutrition and is often only diagnosed by the rapid disappearance of symptoms on administering an antiscorbutic.

EXPERIMENTAL SCURVY.—It is generally considered that scurvy in guinea-pigs is practically identical with human scurvy. No doubt certain differences exist, but it is believed that these may be attributed to concurrent bacterial infection, increased susceptibility to infection being in itself a characteristic feature of scurvy in animals.

REQUIREMENTS OF ANIMALS FOR VITAMIN C.—The fact that some animals, notably the rat, seem to be able to live almost without vitamin C has given rise to much discussion as to whether different animals can store or synthesise vitamin C within the body.

Harden and Zilva (1920) have suggested that, while the metabolic requirement of vitamin C of the monkey and the guinea-pig may be the same, the monkey may have a higher store of the antiscorbutic factor, while Parsons (1920) has advanced the theory that the rate of excretion may be different in different animals, producing less waste of the vitamin. Another suggestion is that of Thurston, Palmer, and Eckles (1929) that synthesis of vitamin C may take place in the adrenals of certain animals, but not in those of the guinea-pig or man.

PATHOLOGICAL CHANGES IN EXPERIMENTAL SCURVY.—Until very recently the conclusion derived from examination of the bone lesions in scurvy was that there was no actual decalcification, the changes being those of osteoblastic degeneration. The studies of Salter and Aub (1931), however, appear to show that the deposition of bone salts in the epiphysis and trabecular tissue is deficient, and that during the process of healing under the administration of antiscorbutic agents normal deposition is restored.

The most important of the changes noted in other structures consist in the degeneration of intercellular "cement" substance, a "liquefaction" (Meyer and McCormick) of the cytoplasm and cell walls upon which depends the hæmorrhage which is an essential lesion of scurvy. Here again arises the question of maintenance of normal cell permeability as a characteristic feature of vitamin action. The changes in the teeth are so characteristic that upon them has been based Höjer's method for the determination of vitamin C in foodstuffs. According to Höjer, the essential lesion takes place in the odontoblast layer, a change, in fact, of the odontoblasts into osteoblasts, so that bone is formed in abnormal positions, particularly the denture and pre-denture layers.

LOWERED RESISTANCE TO INFECTION IN VITAMIN C DEFICIENCY.—Although the power of conferring increased resistance to infection cannot be regarded as a special attribute of vitamin C, as it is of vitamin A, there is no doubt that animals suffering from scurvy do show an increased susceptibility to infection. So definite is this tendency that it is considered to account for many variations in the symptom picture of experimental scurvy. Findlay regards it as a possibly specific consequence of the degeneration of bone marrow found in scurvy, while Werkman and co-workers are inclined to attribute it to the lowered phagocytic and bactericidal activity produced by the lowered body temperature characteristic of most avitaminoses.

VITAMIN C IN FOODSTUFFS.—One of the most interesting facts emerging from the examination of various foodstuffs for their antiscorbutic potency is that considerable differences are found in this respect even among species so closely allied as the citrus fruits. That lime juice is by no means so efficacious as lemon juice was clearly demonstrated by the investigations of Henderson Smith (1918, 1919), her results providing an explanation for the fact that the "lemon juice" (in reality lime juice) used in many expeditions until the middle of the nineteenth century failed to prevent scurvy.

The canning of fruits and vegetables has lately received close scrutiny from the point of

view of their vitamin C content, and the distrust with which these products were once viewed has been greatly allayed by the results obtained by Eddy, Kohman, and co-workers. According to their results, fruits and vegetables which are gathered in prime condition and canned by modern processes, without unnecessary storage, not only suffer no loss of vitamin content but are in fact richer than the same fruits of an inferior class and undergoing deterioration from storage. The drying and preservation of fruits and vegetables is apparently less satisfactory.

Milk is variable in its antiscorbutic content, being to some extent dependent on the diet of the cow or nursing mother. According to the estimate of Hess (1920), one pint of fresh milk of average antiscorbutic potency (*i.e.* undiminished by heating, long keeping, or faulty maternal diet) should provide sufficient antiscorbutic vitamin for an infant. Dried milk is antiscorbutic only so far as the process of drying is carried out rapidly and in the absence of oxygen.

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PLATES 1-7

HISTOLOGICAL CHANGES IN THE VISCERA DUE TO VITAMIN DEFICIENCY.

(See Text, pp. 36 and 43.)

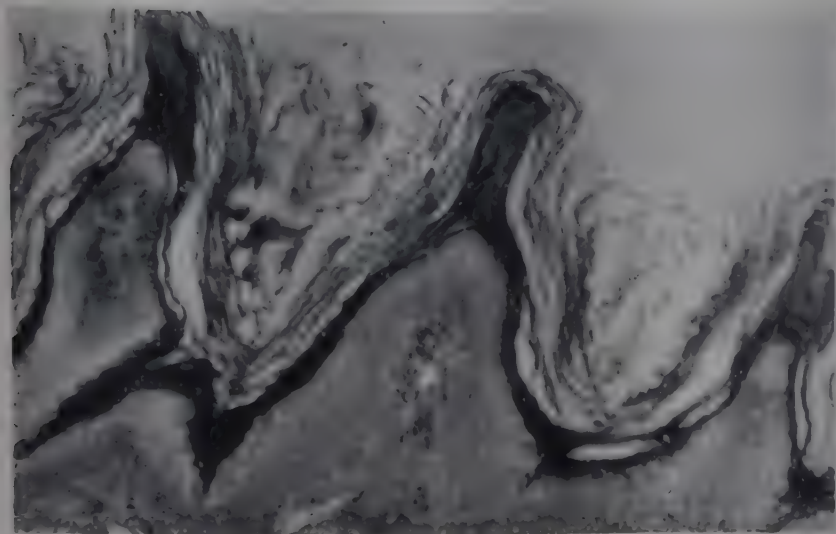


FIG. 1.

Photomicrograph of part of a villiform papillomatous outgrowth in proximal portion of the stomach of a rat fed on the "bad diet" referred to in the text. $\times 400$.

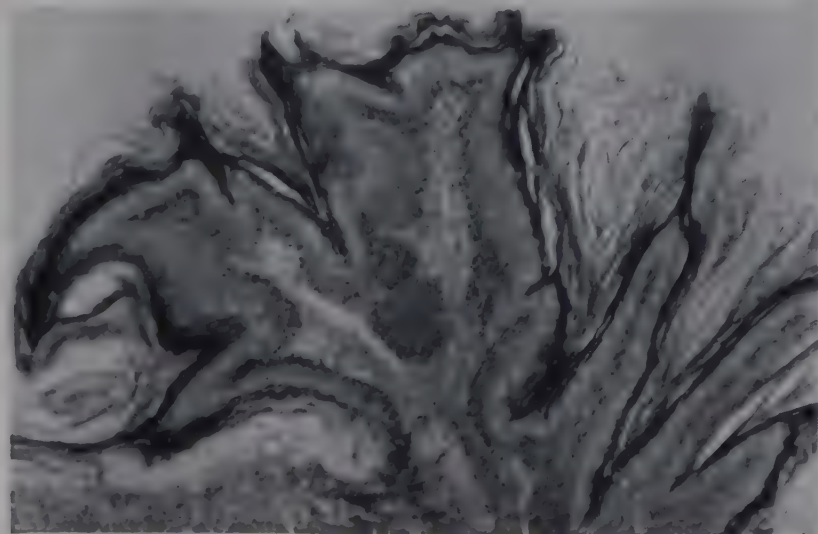


FIG. 2.

Photomicrograph of part of a villiform papillomatous outgrowth found in proximal portion of the stomach of a rat fed on the "bad diet" referred to in the text. $\times 400$.

(After McCarrison, *Brit. Med. Journ.*, October 23, 1926. "A Good Diet and a Bad One.")

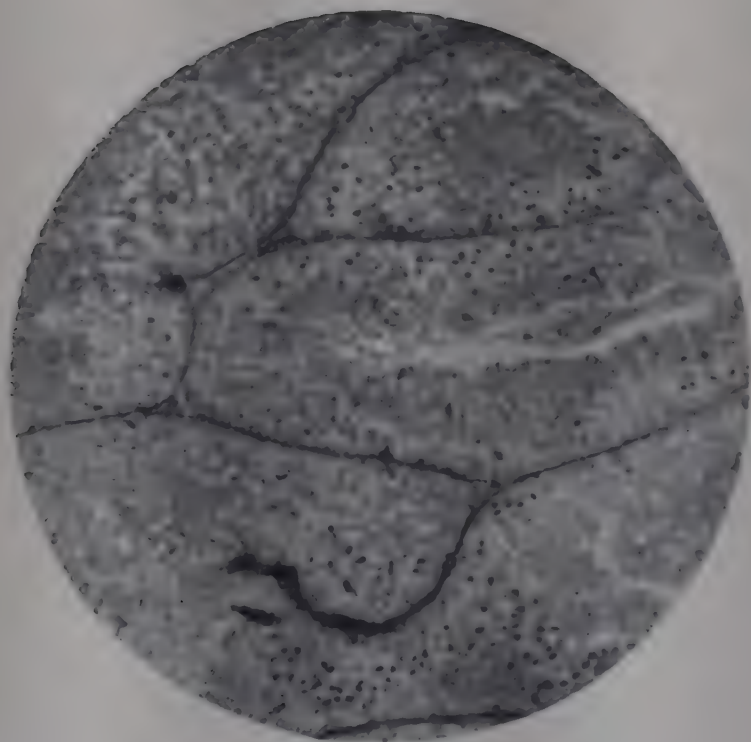


FIG. 4.

Normal testicle of pigeon. $\times 95$.

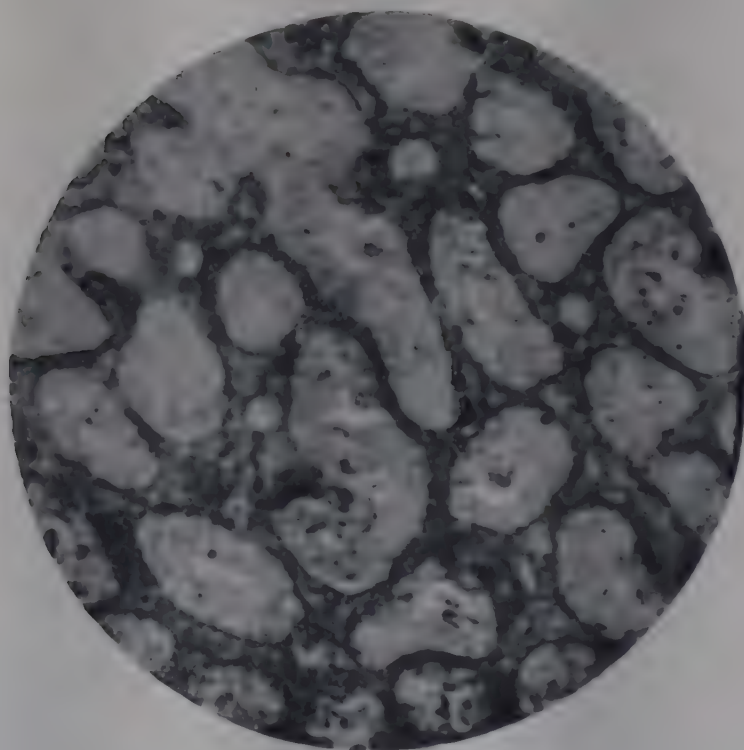


FIG. 5.

Testicle of pigeon ($\times 95$), showing the effect of a diet deficient in accessory food factors. Note narrowing of tubules, great thickening of intertubular tissue, and almost complete absence of sperm elements.

(After McCarrison, *Indian Journ. of Med. Research* (1919), Vol. 6, No. 3.)

INTESTINAL LESIONS DUE TO VITAMIN A DEFICIENCY.

(See Text, p. 92.)

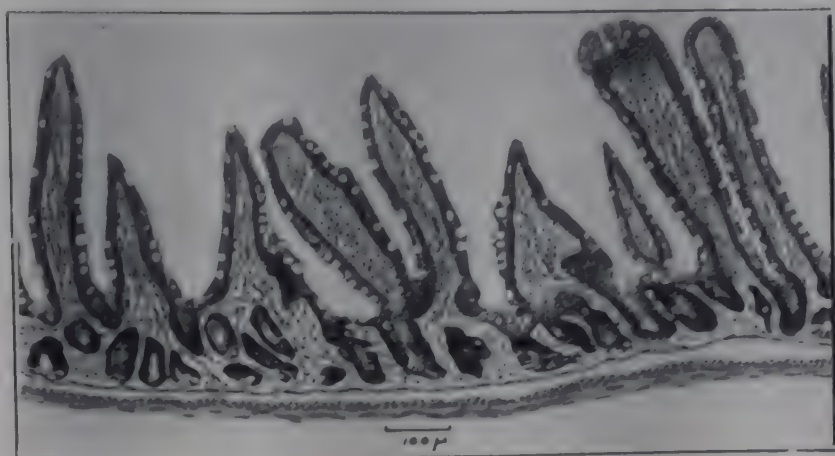


FIG. 1.

Small intestine of rat (longitudinal section) kept on a vitamin-rich diet. Fixed in formol saline. $\times 60$.

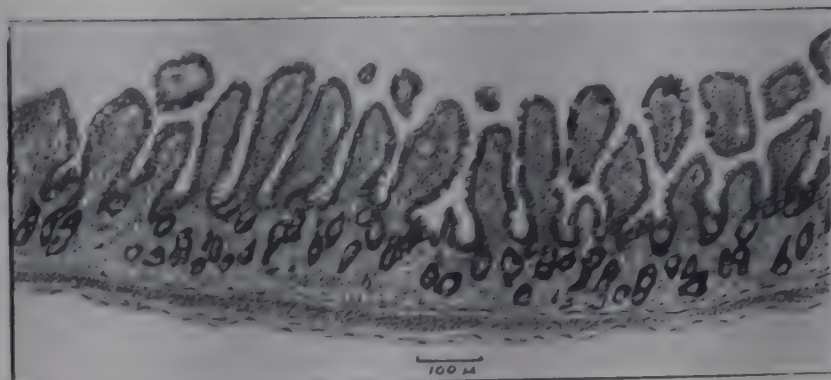


FIG. 2.

Small intestine of rat (longitudinal section) kept on a diet free from vitamin A.



FIG. 3.

Small intestine of rat (longitudinal section) kept on a diet free from vitamin A.

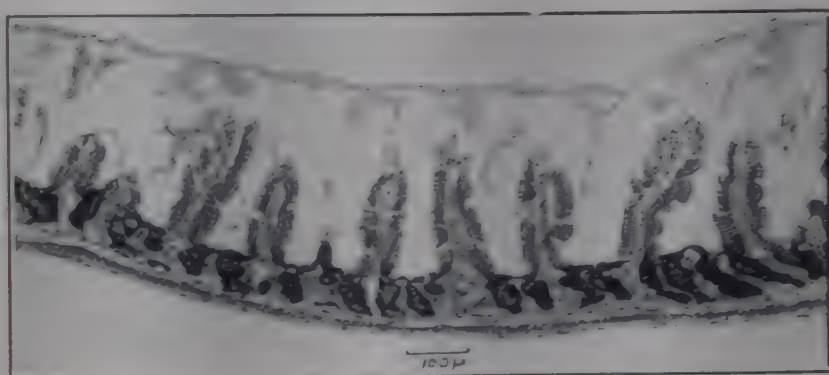


FIG. 4.

Small intestine of rat (longitudinal section) kept on a diet free from vitamin A for five weeks. Formol saline. $\times 60$.



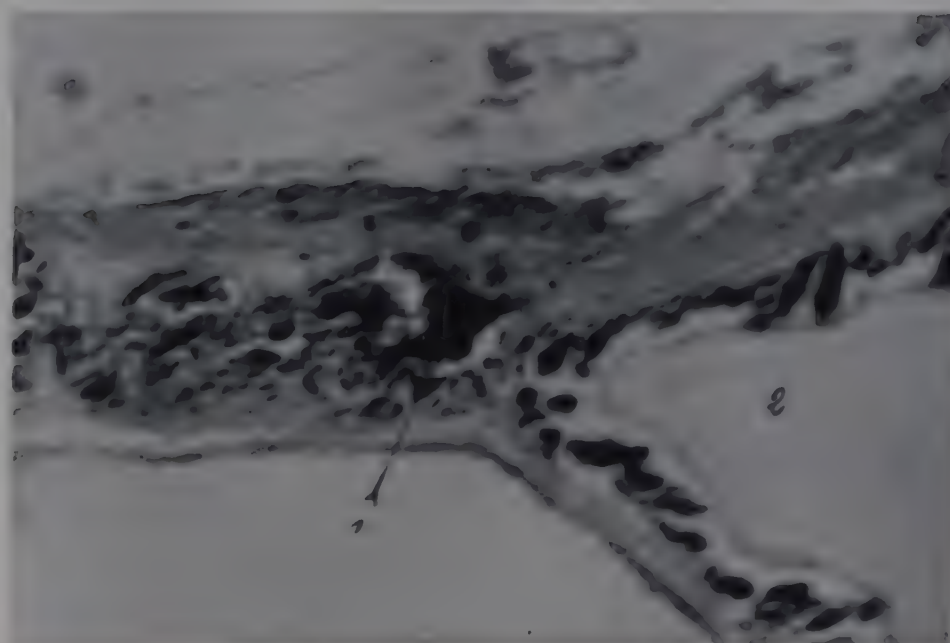
FIG. 5.

Mucous glands of caecum of rat kept on a diet free from vitamin A. Formol saline stained with Murray's Nile-blue-van Gieson method for bacteria, which are seen filling the lumen of the glands cell division on wall of distended gland.

(After Cramer. Reproduced from *Lancet*, 1923, i. p. 1046.)

HYPERVITAMINOSIS D.

(See Text, p. 178.)



Section of Aorta in Hypervitaminosis D.

- (1) Groups of degenerated cells impregnated with calcareous salts.
- (2) Area of necrosis.

(After Jacqumin and Ledoux, 1929, *Revue Belge de Sci. Med.*, Vol. 1.)

VITAMIN D AND CALCIFICATION OF TEETH.

(See Text, p. 186.)

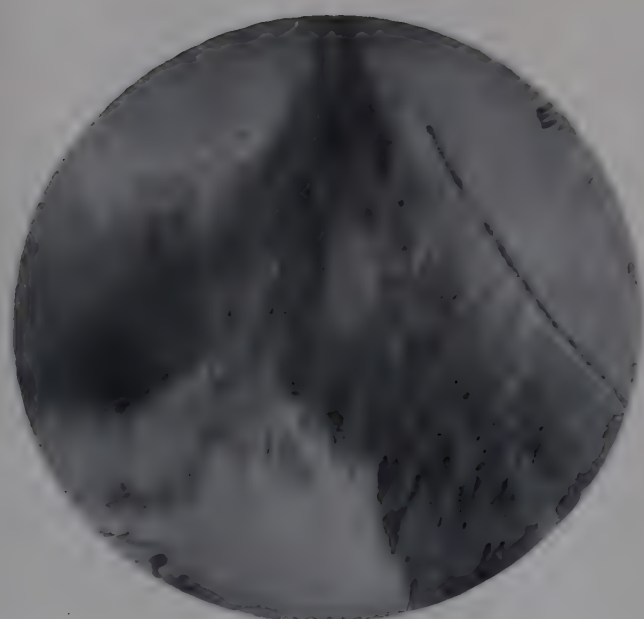


FIG. 1.

Photomicrograph of ground section of a perfectly formed human deciduous molar. Rarely found.

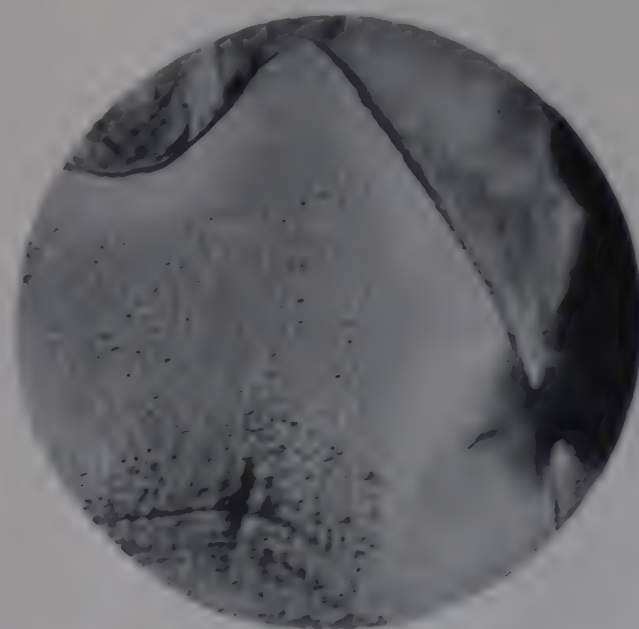


FIG. 2.

Photomicrograph of human deciduous molar. Note defect in structure of dentine. A typical specimen as ordinarily found in this country.

(After May Mellanby, *Brit. Med. Journ.*, March 20, 1926.)

VITAMIN D AND CALCIFICATION OF TEETH.

(See Text, p. 186.)

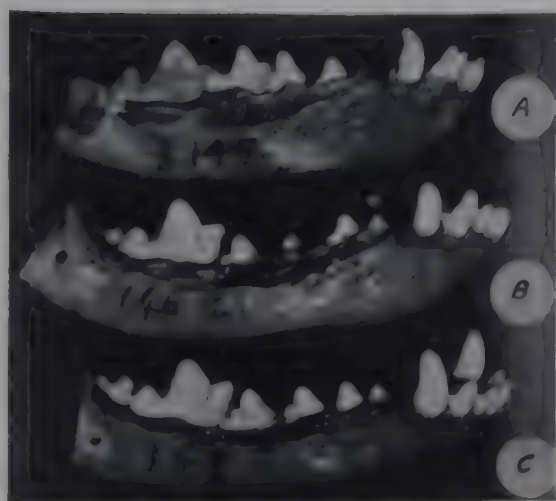


FIG. 3 (A, B, and C.)

The effect of the calcifying vitamin. The jaws of three puppies of the same litter brought up on the same diets except that A contained 10 c.c. of linseed oil daily, B contained 10 grms. of butter daily, and C contained 10 c.c. of cod-liver oil daily. Note the perfect formation of the teeth of C and the imperfectly formed teeth in A.

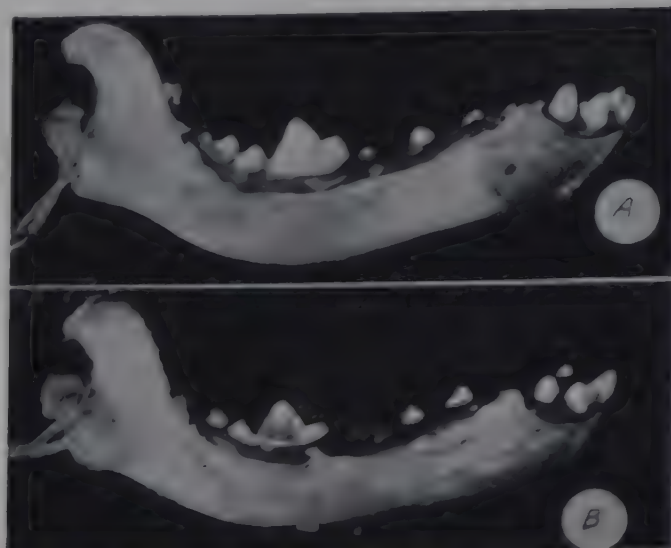
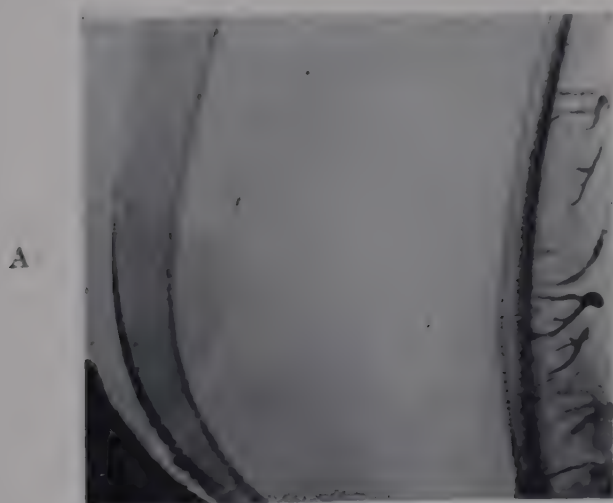
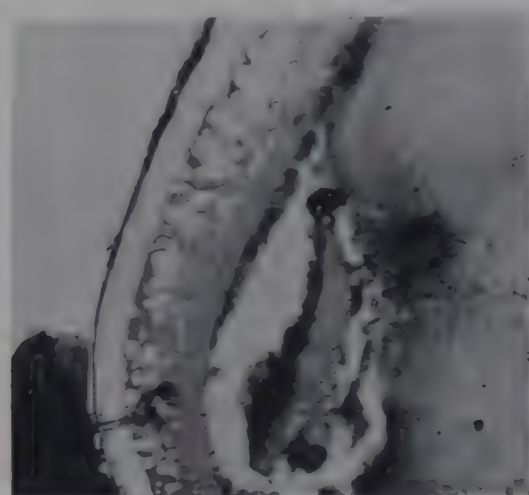


FIG. 5 (A and B).

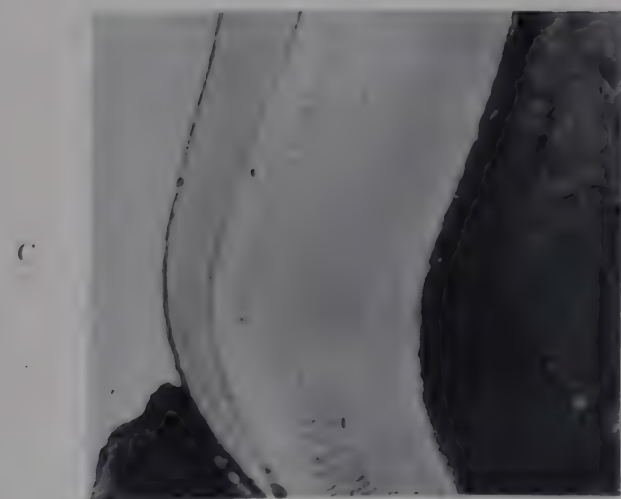
The effect of ultra-violet radiations. Photographs of the lower jaws of two puppies brought up on the same diet deficient in calcifying vitamin and living under the same conditions. Puppy A only was exposed thrice weekly for 20 minutes to the radiations of a mercury-vapour lamp. Note the better formed teeth of A as compared with B.



A



B



C



D

FIG. 4 (A, B, C, and D).

The effect of different cereals. Photomicrographs of ground sections of molar teeth of four puppies of the same litter. The diets of B, C, and D were deficient in antirachitic vitamin and were identical except that B contained oatmeal as cereal, C contained white flour as cereal, and D contained white flour and wheat germ (10 per cent.) as cereal. The diet of A was identical with that of B—that is, it contained oatmeal as cereal, but olive oil in diet B was replaced by 10 c.c. of cod-liver oil, which completely antagonised the bad effect of the oatmeal. Note how defective is the dentine in B (oatmeal), also that wheat germ has made the teeth of D worse than C (white flour). (After May Mellanby, *Brit. Med. Journ.*, March 20, 1926.)

INFECTIVE THEORY OF BERI-BERI.

(See Text, p. 244.)



FIG. 1.

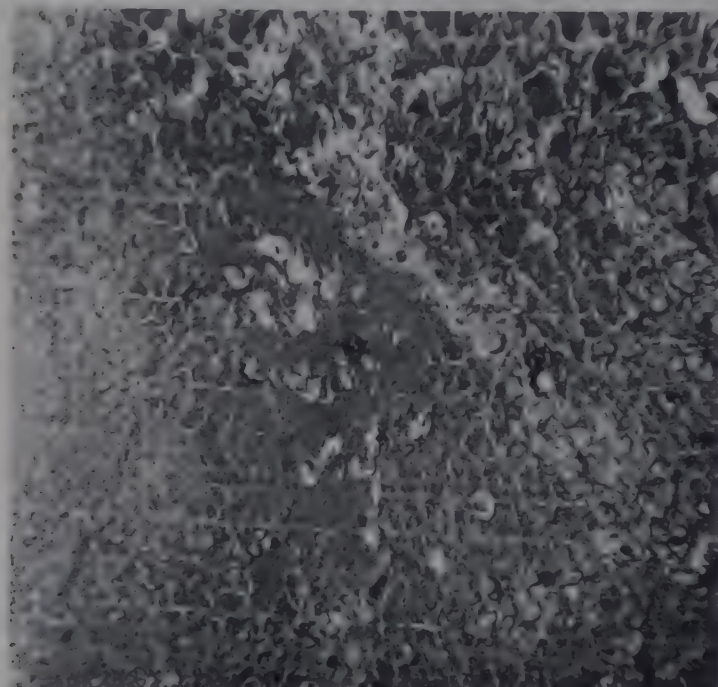
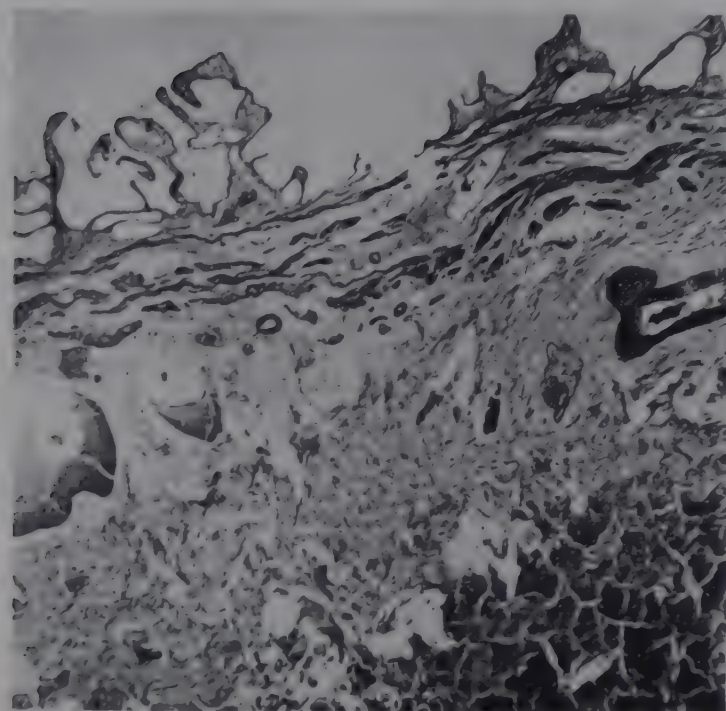
The *Bacillus asthenogenes* of Bernard. Bouillon cultures
12 hours old (anaerobic and aerobic).

(After A. Cannon.)

(Reproduced from *Brit. Med. Journ.*, Nov. 9, 1929.)

CEDEMA OF THE GALL-BLADDER IN BERI-BERI.

(See Text, p. 253.)



FIGS. 2-4.

Transverse section of whole of gall-bladder from
mucous surface above to liver below, showing intense
œdema. $\times 25$.

(After Tull, *Trans. Roy. Soc. Trop. Med. and Hyg.*,
1925, Vol. 22.)

THE SPLEEN IN VITAMIN B₁ DEFICIENCY.

(See Text, p. 272.)

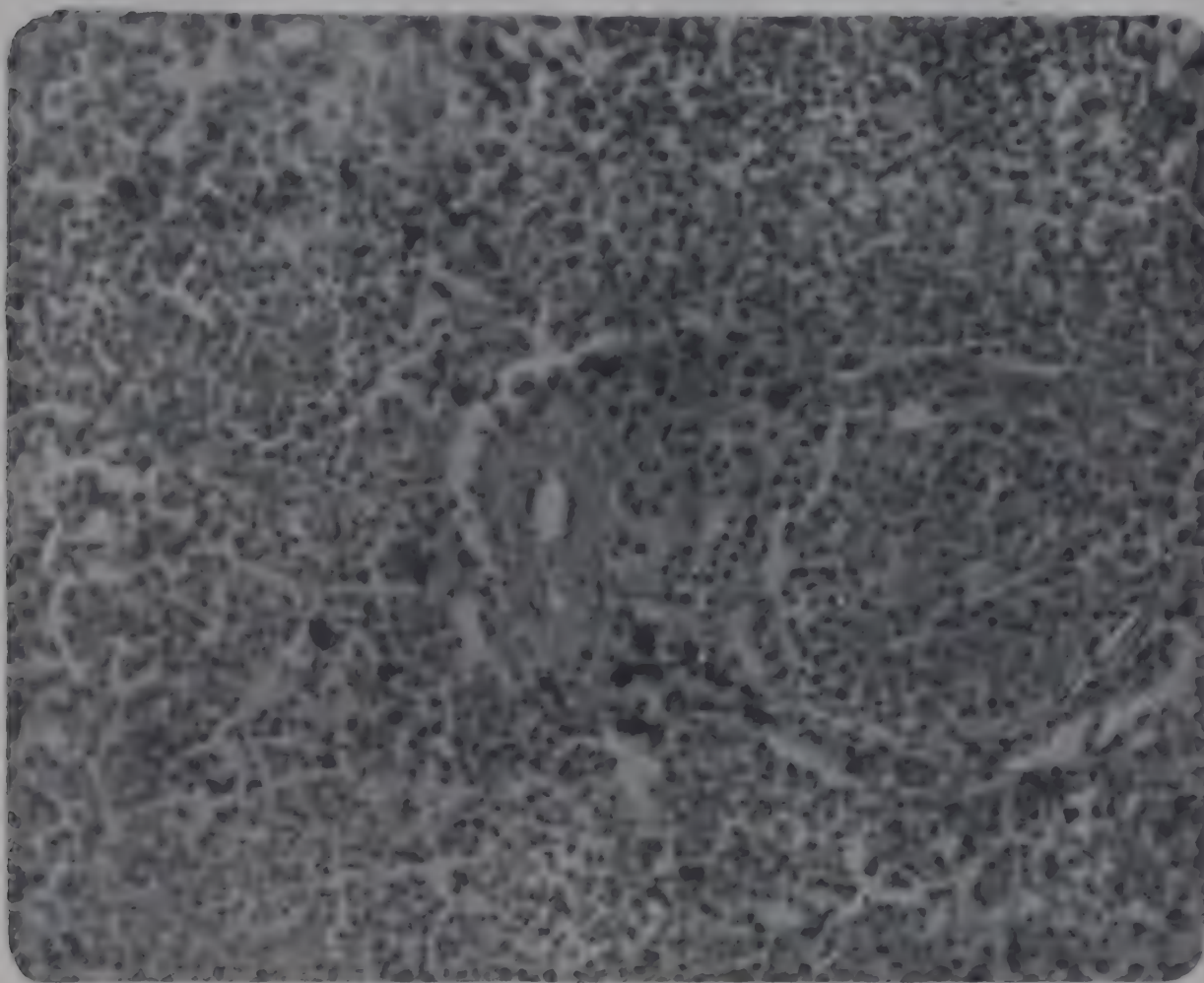


FIG. 1.
Spleen. Normal Pigeon

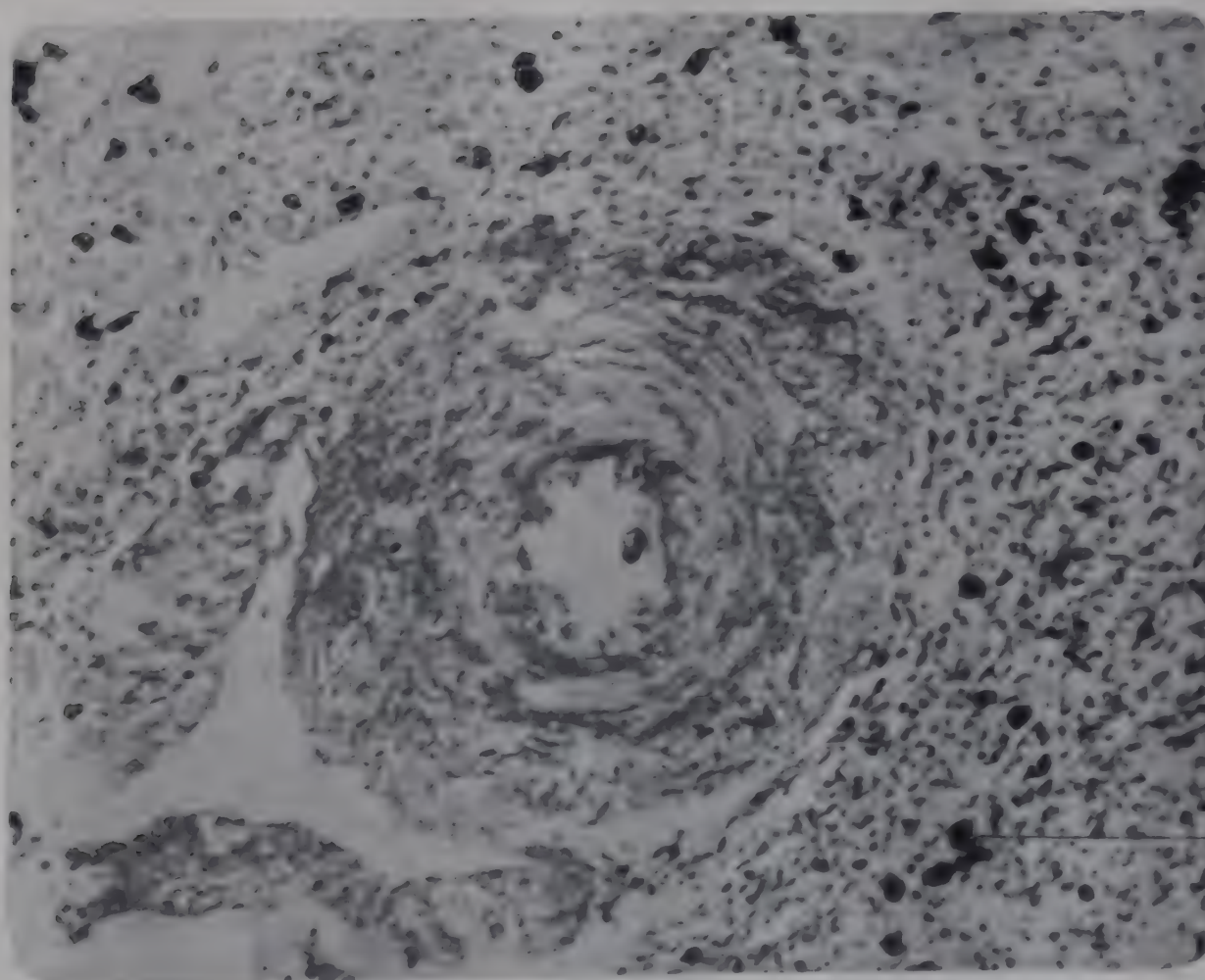
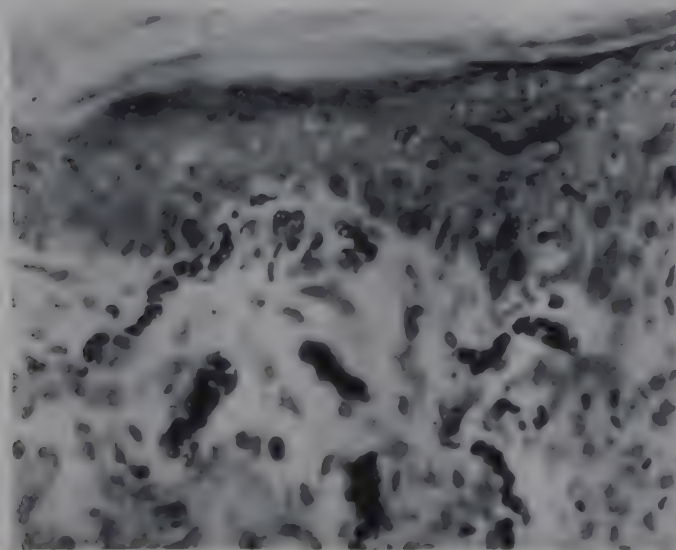


FIG. 2.
Spleen. Polyneuritis avium.

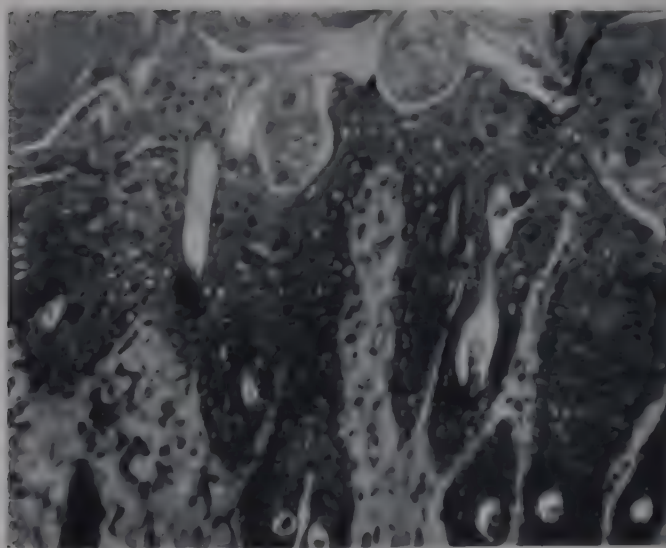
(After McCarrison. *Pathogenesis of Deficiency Diseases*, No. V.)

LESIONS IN VITAMIN B₂ DEFICIENCY.

(See Text, pp. 308 and 309.)



Skin from chest of rat on vitamin B₂ deficiency diet. Early changes; congestion of vessels in dermis.



Skin from chest of rat on vitamin B₂ deficient diet. Hyperkeratosis and round-celled infiltration in dermis.

(After G. M. Findlay, 1928, *Journ. Path. and Bact.*, Vol. 31.)



Cells of anterior horn of spinal cord of rat on vitamin B₂ deficient diet.

(After Stern and Findlay, 1929, *Journ. Path. and Bact.*, Vol. 32.)

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